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(54) **BIODEGRADABLE SUPERPARAMAGNETIC MATERIALS USED IN CLINICAL APPLICATIONS**

BIODEGRADIERBARES SUPERPARAMAGNETISCHES MATERIAL ZUR VERWENDUNG IN KLINISCHEN ANWENDUNGEN

MATERIAU SUPER PARAMAGNETIQUE BIODEGRADABLE UTILISE DANS DES APPLICATIONS CLINIQUES

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Description

This application is a continuation-in-part of the applicants' prior co-pending U.S. Application Serial No. 882,044 filed July 3, 1986.

1. INTRODUCTION

This invention relates to materials exhibiting certain magnetic and biological properties which make them uniquely suitable for use as magnetic resonance imaging (MRI) agents to enhance MR images of animal organs and tissues. More particularly, the invention relates to the in vivo use of biologically degradable and metabolizable superparamagnetic metal oxides as MR contrast agents. Depending on their preparation, these metal oxides are in the form of superparamagnetic particle dispersoids or superparamagnetic fluids where the suspending medium is a physiologically-acceptable carrier. These dispersoids and fluids are administered to animals, including humans, by a variety of routes and the metal oxides therein collect in specific target organs to be imaged. The biodistribution of the metal oxides in target organs or tissues results in a more detailed image of such organs or tissues because the metal oxides, due to their superparamagnetic properties, exert profound effects on the hydrogen nuclei responsible for the MR image. In addition, the dispersoids and fluids are quite stable and, in the case of the fluids, can even be subjected to autoclaving without impairing their utility. Thus, the materials are well-suited for in vivo use.

The combination of superparamagnetism and biodegradability makes the materials described herein particularly advantageous for use as MR contrast agents. Superparamagnetism, which results in profound capabilities to alter MR images, makes it possible to use these materials in concentrations lower than those required for MRI with other types of magnetic materials. Biodegradability results in optimum retention times within the organs and tissues to be imaged, i.e., the materials remain within the organ or tissue sufficiently long to permit an image to be obtained, yet are eventually cleared from or metabolized within the organ or tissue. Remarkably, when iron-based agents are administered, the iron thereon is eventually metabolized and incorporated into the subject's hemoglobin.

These materials can, therefore, be used for a variety of clinical diagnostic purposes including, but not limited to, detection of cancerous lesions in liver and other reticuloendothelial tissue, detection of cancerous or other lesions in the intestine, detection of liver diseases, such as cirrhosis and hepatitis, and assessment of liver regeneration. Those that are iron-based are also clinically useful as anti-anemia agents.

2. BACKGROUND OF THE INVENTION

2.1 IN VIVO NMR IMAGING: GENERAL CONSIDERATIONS

Nuclear magnetic resonance (NMR) is now widely used for obtaining spatial images of human subjects for clinical diagnosis. Clinical usage of NMR imaging, also called magnetic resonance imaging or, simply, MRI, for diagnostic purposes has been reviewed [see e.g., Rykett, et al., Nuclear Magnetic Resonance, pgs. 157-167 (April, 1982) and T.F. Budinger, et al., Science, pgs. 288-298, (October, 1984)]. Several advantages of using such a procedure over currently used diagnostic methods, e.g., x-ray computer-aided tomography (CT), are generally recognized. For instance, the magnetic fields utilized in a clinical NMR scan are not considered to possess any deleterious effects to human health (see Budinger, supra., at 296). Additionally, while x-ray CT images are formed from the observation of a single parameter, x-ray attenuation, MR images are a composite of the effects of a number of parameters which are analyzed and combined by computer. Choice of the appropriate instrument parameters such as radio frequency (Rf), pulsing and timing can be utilized to enhance (or, conversely, attenuate) the signals of any of the image-producing parameters thereby improving the image quality and providing better anatomical and functional information. Finally, the use of such imaging has, in some cases, proven to be a valuable diagnostic tool as normal and diseased tissue, by virtue of their possessing different parameter values, can be differentiated in the image.

In MRI, the image of an organ or tissue is obtained by placing a subject in a strong external magnetic field and observing the effect of this field on the magnetic properties of the protons (hydrogen nuclei) contained in and surrounding the organ or tissue. The proton relaxation times, termed T_1 and T_2, are of primary importance. T_1 (also called the spin-lattice or longitudinal relaxation time) and T_2 (also called the spin-spin or transverse relaxation time) depend on the chemical and physical environment of organ or tissue protons and are measured using the Rf pulsing technique; this information is analyzed as a function of distance by computer which then uses it to generate an image.

The image produced, however, often lacks definition and clarity due to the similarity of the signal from other tissues. To generate an image with good definition, T_1 and/or T_2 of the tissue to be imaged must be distinct from that of the background tissue. In some cases, the magnitude of these differences is small, limiting diagnostic effectiveness. Thus, there exists a real need for methods which increase or magnify these differences. One approach is the use of contrast agents.

2.2. MRI CONTRAST AGENTS

As any material suitable for use as a contrast agent must affect the magnetic properties of the surrounding tissue, MRI contrast agents can be categorized by their magnetic properties.

Paramagnetic materials have been used as MRI contrast agents because of their long recognized ability to decrease T_1 [Weinmann et al., Am. J. Rad. 142, 619 (1984), Greif et al. Radiology 157, 461 (1985), Runge, et al. Radiology 147, 789 (1983), Brasch, Radiology 147, 781 (1983)]. Paramagnetic materials are characterized by a weak, positive magnetic susceptibility and by their inability to remain magnetic in the absence of an applied magnetic field.

Paramagnetic MRI contrast agents are usually transition metal ions of iron, manganese or gadolinium. They may be bound with chelators to reduce the toxicity of the metal ion (see Weinman reference above). Paramagnetic materials for use as MRI contrast agents are the subject of a number of patents and patent applications. (See EPA 0 160 552; UK Application 2 137 612A; EPA 0 184 899; EPA 0 186 947; US Patent 4,615,879; PCT WO 85/05554; and EPA 0 210 043).

Ferromagnetic materials have also been used as contrast agents because of their ability to decrease T_2 [Medonca-Dias and Lauterbur, Magn. Res. Med. 3, 328, (1986); Olsson et al., Mag Res. Imaging 4, 437 (1986); Renshaw et al. Mag Res. Imaging 4, 351 (1986) and 3, 217 (1986)]. Ferromagnetic materials have high, positive magnetic susceptibilities and maintain their magnetism in the absence of an applied field. Ferromagnetic materials for use as MRI contrast agents are the subject of recent patent applications [PCT WO 86/01112; PCT WO 85/04330].

A third class of magnetic materials termed superparamagnetic materials have been used as contrast agents [Saini et al Radiology, 167, 211 (1987); Hahn et al., Soc. Mag Res. Med. 4(22) 1537 (1986)]. Like paramagnetic materials, superparamagnetic materials are characterized by an inability to remain magnetic in the absence of an applied magnetic field. Superparamagnetic materials can have magnetic susceptibilities nearly as high as ferromagnetic materials and far higher than paramagnetic materials [Bean and Livingston J. Appl. Phys. suppl to vol. 30, 1205, (1959)].

Ferromagnetism and superparamagnetism are properties of lattices rather than ions or gases. Iron oxides such as magnetite and gamma ferric oxide exhibit ferromagnetism or superparamagnetism depending on the size of the crystals comprising the material, with larger crystals being ferromagnetic [G. Bate in Ferromagnetic Materials. vol. 2, Wohlfarth (ed.) p.439].

As generally used, superparamagnetic and ferromagnetic materials alter the MR image by decreasing T_2 resulting in image darkening. When injected, crystals of these magnetic materials accumulate in the targeted organs or tissues and darken the organs or tissues where they have accumulated. Abnormal volumes of liver, such as tumors, are deficient in their ability to take up the magnetic materials and appear lighter against normal background tissue than they would without contrast agent.

2.3 SUPERPARAMAGNETIC MATERIALS

As stated supra, superparamagnetic materials possess some characteristics of paramagnetic and some characteristics of ferromagnetic materials. Like paramagnetic materials, superparamagnetic materials rapidly lose their magnetic properties in the absence of an applied magnetic field; they also possess the high magnetic susceptibility and crystalline structure found in ferromagnetic materials. Iron oxides such as magnetite or gamma ferric oxide exhibit superparamagnetism when the crystal diameter falls significantly below that of purely ferromagnetic materials.

For cubic magnetite (Fe_3O_4) this cut-off is a crystal diameter of about $300 \cdot 10^{-10}$ m (300 angstroms)[Dunlop, J. Geophys. Rev. 78 1780 (1972)]. A similar cut-off applies for gamma ferric oxide [Bare in Ferromagnetic Materials, vol. 2, Wohfarth (ed.) (1980) p. 439]. Since iron oxide crystals are generally not of a single uniform size, the average size of purely ferromagnetic iron oxides is substantially larger than the cut-off of $300 \cdot 10^{-10}$ m (300 angstroms). For example, when gamma ferric oxide is used as a ferromagnetic material in magnetic recording, (e.g. Pfizer Corp. Pf 2228), particles are needle-like and about $0,35 \cdot 10^{-6}$ m (0.35 microns) long and $0,06 \cdot 10^{-6}$ m (0.06 microns) thick. Other ferromagnetic particles for data recording are between 0,1 and $10 \cdot 10^{-6}$ m (0.1 and 10 microns) in length [Jorgensen, The Complete Handbook of Magnetic Recording, p. 35 (1980)]. For a given type of crystal, preparations of purely ferromagnetic particles have average dimensions many times larger than preparations of superparamagnetic particles.

The theoretical basis of superparamagnetism has been described in detail by Bean and Livington [J. Applied Physics, Supplement to volume 30, 1205 (1959)]. Fundamental to the theory of superparamagnetic materials is the destabilizing effect of temperature on their magnetism. Thermal energy prevents the alignment of the magnetic moments present in superparamagnetic materials. After the removal of an applied magnetic field, the magnetic moments of superparamagnetic materials still exist, but are in rapid motion, causing a randomly oriented or disordered magnetic moment and, thus, no net magnetic field. At the temperatures of biological systems and in the applied magnetic fields of MR imagers, superparamagnetic materials are less magnetic than their ferromagnetic counterparts. For example, Berkowitz et al. [(J. App. Phys. 39, 1261 (1968)] have noted decreased magnetism of small superparamagnetic iron oxides at elevated temperatures. This may in part explain why workers in the field of MR imaging have looked to ferromagnetic materials as contrast agents on the theory that the more magnetic a material is per gram, the more effective

that material should be in depressing T_2 [Drain, Proc. Phys. Soc. 80, 1380 (1962); Medonca-Dias and Lauterur, Mag. Res. Med. 3, 328 (1986)].

2.4. WATER-BASED SUPERPARAMAGNETIC SOLUTIONS

It has been recognized for some time that superparamagnetic particles can be fashioned into magnetic fluids termed ferrofluids [see Kaiser and Miskolczy, J. Appl. Phys. 41 3 1064 (1970)]. A ferrofluid is a solution of very fine magnetic particles kept from settling by Brownian motion. To prevent particle agglomeration through Van der Waals attractive forces, the particles are coated in some fashion. When a magnetic field is applied, the magnetic force is transmitted to the entire volume of liquid and the ferrofluid responds as a fluid, i.e. the magnetic particles do not separate from solvent.

Another approach to synthesizing water-based magnetic compounds is disclosed by Gable et al (U.S. Patent No. 4,001,288). Here, the patent discloses that magnetite can be reacted with a hydroxycarboxylic acid to form a water soluble complex that exhibits ferromagnetic behavior both in the solid form and in solution.

2.4.1. PROBLEMS MANIPULATING AQUEOUS SOLUTIONS OF SUPERPARAMAGNETIC MATERIALS

Approaches to the synthesis of aqueous fluids of superparamagnetic iron oxides often involve surrounding iron oxide crystals with polymer or surfactants in an effort to block the attractive forces between the crystals that promote aggregation. In many cases however, the polymer does not completely coat the oxide and the resultant material maintains much of the sensitivity to clumping or aggregation characteristic of the uncoated iron oxide. The tendency to clump, and other peculiar properties of iron oxide solutions, hamper the manipulations of these solutions needed in pharmaceutical manufacture.

The manufacture of a magnetic pharmaceutical solution such as an MRI contrast agent requires an extremely stable solution so certain manipulations, common in pharmaceutical manufacture, can be carried out. Solution stability is defined as the retention of the size of the magnetic material in solution; in an unstable solution the material will clump or aggregate. Such changes in the size of magnetic material alter its biodistribution after injection, an intolerable situation for an MRI contrast agent. A high degree of stability is required to perform common operations associated with pharmaceutical manufacture such as dialysis, concentration, filtration, centrifugation, storage of concentrates prior to bottling, and long term storage after bottling. Particular problems are posed by the need to sterilize aqueous solutions of metal oxide, e.g. iron oxide, for pharmaceutical use.

Additionally, concentrated solutions of aqueous superparamagnetic materials cannot be sterilized by filtration even when the solution is comprised of materials smaller than the pore of the filter. This phenomena is related to the concentration of the solution, for dilute solutions can be filter sterilized. Filter-sterilized, dilute material can be reconcentrated and dispensed into sterile bottles, but such operations offer many chances to recontaminate the product. Autoclaving solutions of superparamagnetic materials after bottling is preferable, since sterilization is achieved after final bottling, and there is little opportunity for contamination of the final product. Autoclaving involves heating sealed solutions to 121°C for 30 minutes. Such extreme temperatures induce aggregation or clumping of the superparamagnetic oxides, making them unusable as an injectable material.

2.5. PARAMAGNETIC FERRIC OXIDES

Paramagnetic iron oxides or ferric oxides are currently used in the treatment of anemia under many trade names such as Imferon®. When dissolved in aqueous solution, such materials can be represented as FeO:OH and are termed ferric oxyhydroxides. They are paramagnetic and exert small, if any, effects of proton relaxivity. They are stable, undergo the manipulations discussed supra for pharmaceutical manufacture, and are commercially available as drugs used in the treatment of anemia.

3. NOMENCLATURE

The term "biodegradable" in reference to the materials of this invention is defined as being metabolized and/or excreted by the subject within 30 days or less; for superparamagnetic iron oxides, the term is further defined as being incorporated in the hemoglobin of a subject within 30 days or less after administration.

The term "blocking agent" is defined as any material which when administered parenterally to a subject, will competitively bind to the receptors of the cells of the reticuloendothelial system which recognize and bind MRI contrast agents.

The term "superparamagnetic fluid" defines any metal oxide fluid produced by the methods described in section 6.3 herein, which has the characteristics described in section 6.4 herein.

4. SUMMARY OF THE INVENTION

It is an object of this invention to produce fluids comprising superparamagnetic metal oxide particles which possess attributes which make them particularly well suited for use as contrast agents in in vivo MR imaging techniques. Spe-
5 cifically, it is an object of this invention to provide fluids which contain particles that are easily administered, exert a significant effect on the image produced and which distribute in vivo to specific organs or tissues. It is further an object of this invention to provide means whereby the particles comprising these fluids can be directed, in vivo, to a specific target organ or tissue.

The fluids of the instant invention meet all of these objectives. These fluids are stable in vivo, can be easily proc-
10 essed for in vivo use, and overcome problems of toxicity and excessively long retention in the subject (i.e., are biodegradable). Remarkably, it has been found that the fluids of this invention are quite stable and can be prepared well in advance of use and stored. In addition, the particles comprising these fluids surprisingly exert a greater effect on T_2 than ferromagnetic or paramagnetic materials, thereby producing a well-resolved, negative contrast image of an in vivo target organ or tissue.

15 Moreover, it has also been surprisingly found that the particles comprising these fluids exhibit highly desirable in vivo retention times, i.e., they remain intact for a sufficient time to permit the image to be taken, yet are ultimately biodegradable. Remarkably, once degraded, iron-based particles serve as a source of nutritional iron. Additionally, the particles are sufficiently small to permit free circulation through the subject's vascular system and rapid absorption by the organ/tissue being imaged, allowing for maximum latitude in the choice of administration routes and ultimate targets.

20 In this context, the instant superparamagnetic particles overcome problems associated with the use of ferromagnetic and paramagnetic materials as MR contrast agents. Specifically superparamagnetic particles, because they are smaller than ferromagnetic particles, are more able to avoid uptake by the subject's reticuloendothelial cells and may be more effectively targeted to other organ and tissue sites within the body. Also, because the superparamagnetic metal oxide particles are smaller than ferromagnetic particles, they have higher surface area per unit mass and are more eas-
25 ily and rapidly digested by chemical or metabolic processes. However, the superparamagnetic particles used herein, because they are larger than paramagnetic ions, are not so rapidly metabolized in the target organ or tissue as to prevent convenient imaging.

The superparamagnetic fluids are produced by a unique four step process from a mixture of divalent and trivalent salts, such as $FeCl_2$ and $FeCl_3$. In addition, this process permits incorporation of other metals similar to iron (such as
30 cobalt (Co) and manganese (Mn)) into the fluids by replacing some of the divalent iron salts with divalent salts of these metals. In the process, the salts are precipitated in base to form corresponding metal oxide particles. These particles are then dispersed and oxidized by sonication of the mixture; the result, remarkably, are superparamagnetic ferric oxyhydroxide particles. Insoluble particles are then removed by centrifugation and the final fluid is dialyzed against a neutral or alkaline buffer suitable for in vivo use.

35 In a preferred embodiment, the salt mixture is $FeCl_2/FeCl_3$ in a 1:2 ratio, which leads to the production of ferric oxyhydroxide particles, and the buffer is 10 mM ammonium citrate at pH 8.2. The resulting superparamagnetic fluid is highly stable and can withstand autoclaving.

The superparamagnetic fluids exhibit some of the magnetic properties of superparamagnetic ferrofluids (e.g., the particles in them cannot be removed from solution by magnetic manipulation). When dispersed in these fluids, the
40 superparamagnetic particles will not scatter visible light, indicating that the individual metal oxide "particles" are quite small (generally between 50 and 4000 · 10^{-10} m in diameter).

The superparamagnetic particles of the invention exist in the bulk fluid as ionic crystals, having both ionic and crystalline characteristics. In common with magnetite (Fe_3O_4) and gamma ferric oxide (gamma Fe_2O_3), they have high magnetic susceptibility. In common with ionic forms of ferric oxide, the so-called ferric oxyhydroxides, they cause retention
45 of anions. The counterion to the crystals can be any one of a number of organic anions.

The superparamagnetic particles in the fluids comprise crystalline cores which can range from about 50 · 10^{-10} m to 500 · 10^{-10} m in diameter. These cores may optionally be surrounded by a coating comprising a polysaccharide, a protein, a polypeptide, an organosilane, or any composite thereof. By way of illustration, the polysaccharide coating may comprise dextran of varying molecular weights and the protein coating may comprise bovine or human serum albu-
50 min. With coatings, the overall particle diameter can range between about 50 · 10^{-10} m to 5000 · 10^{-10} m.

These polymeric coatings serve as a base to which biological molecules can be attached. Biological molecules can be used to direct the superparamagnetic metal oxide particles to the desired target and are preferentially recognized and bound by the target organ or tissue. Suitable biomolecules include proteins, polysaccharides, hormones, antibodies etc.

55 The use of sub-micron sized superparamagnetic particles is particularly important when the route of administration is intravascular, as such particles can freely circulate in the subject's vascular system, since they are small enough to pass through the capillary network. Thus, such particles can be carried to targeted organs or tissue after being intravascularly administered with a minimum of trouble or delay.

Once administered, the particles within the fluids collect in the target organ or tissue and exert a profound contrast effect to permit an image to be taken. The particles act primarily to enhance T_2 relaxation, but T_1 is also affected (although to a lesser extent).

For example, when the particles are administered invascularly (e.g., intravenously or intra-arterially), they are selectively distributed to reticuloendothelial organs, including the liver, spleen, lymph nodes, bone marrow and, to a lesser extent, the lungs. However, when the particles are administered via the gastrointestinal tract, e.g. orally, by intubation or by enema, they can be used as imaging agents for the organs and tissues of the gastrointestinal tract.

In one embodiment, a fluid containing dextran-coated iron oxide particles can be injected into a subject's bloodstream and the particles localize in the liver. These particles are absorbed by the reticuloendothelial cells of the liver by phagocytic uptake; a particular benefit of this mode of uptake is that phagocytized iron is metabolized and cleared from the liver much more slowly (but not so slowly as to lead to undesirably long retention times) than prior art paramagnetic ions. Additionally, the dextran-coated particles can be preferentially absorbed by healthy-cells, with less uptake into cancerous (tumor) cells. This preferential uptake enhances the contrast between healthy and cancerous tissue and allows for better definition of the tumor location on the image.

5. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graphical representation comparing the effect of ferromagnetic and superparamagnetic contrast agents on T_2;

FIG. 2 is a composite of five in vivo MR images of a cancerous rat liver obtained on a Technicare MR Imager;

FIGS. 2A and 2B were obtained without the use of contrast agents and were taken at different settings of the instrument;

FIGS. 2C and 2D were obtained after the intravenous administration of the dextran-coated particle produced in Example 6.1. at a dosage of 0.5 mg/kg; the tumor can clearly be seen;

FIG. 2E is the image reproduced in FIG. 2C showing the tumor highlighted by crosshairs.

FIG. 3 is a graphical representation of the % T_2 reduction in liver and spleen tissue for three different dosages of an uncoated superparamagnetic particle as a function of time after administration;

FIG. 4 presents hysteresis curves for paramagnetic, ferromagnetic and superparamagnetic iron oxides;

FIG. 5 shows the effect of autoclaving on superparamagnetic fluids having varying concentrations of citrate;

FIG. 6 presents a schematic diagram of the apparatus used in example 7.10; and

FIG. 7 is a graphical representation of the T_2 of rat blood as a function of time after the injection of a dextran-coated superparamagnetic iron oxide particle with and without the use of a blocking agent.

6. DETAILED DESCRIPTION OF THE INVENTION

6.1. PREPARATION OF COATED SUPERPARAMAGNETIC IRON OXIDE PARTICLES

The synthesis of superparamagnetic iron oxide particles for use as MRI contrast agents is accomplished by mixing ferrous and ferric salts with base to form a black, magnetic oxide of iron. Crystals result from such precipitations, for when the material is subjected to X-ray diffraction analyses long range order is apparent. A diameter of about between 50 to 300 · 10^{-10} m (about 50 and 300 angstroms) for such crystals has been calculated although crystals may range in diameter from about 50 · 10^{-10} to 500 · 10^{-10} m (about 50 to 500 angstroms). The iron oxides have correspondingly high surface areas, greater than about 75 m^2/gm.

The presence of ferrous salts prior to base addition insures the formation of a black, crystalline magnetic iron oxide. Without the ferrous ion, paramagnetic ferric oxide gels (noncrystalline materials) result (as described e.g., in U.S. Patent No. 2,885,393). The presence of divalent iron, so essential to the formation of the superparamagnetic material, can then be removed by exposure of the material to oxidizing conditions. Oxidation of the iron to produce a ferric oxide after formation of the crystal does not alter the usefulness of the material as a contrast agent in MRI or the superparamagnetism.

It is to be understood throughout this detailed description, that the use of superparamagnetic iron oxides as MR contrast agents is but one embodiment of the invention and that superparamagnetic oxides of other magnetic metals, e.g., cobalt or gadolinium, may be substituted for iron oxides.

There are two general strategies for the formation of the coated superparamagnetic iron oxide particles suitable for MRI.

1. Synthesis of iron oxide by precipitation in the presence of polymers like dextran, or polyglutaraldehyde or other material. Such syntheses include those described by London et al., U.S. Patent No. 2,870,740, Molday, U.S. Patent No. 4,452,773, Cox et al., Nature, 208, 237 (1965) and Rembaum, U.S. Patent No. 4,267,234; all of which are incorporated herein by reference.

2. Synthesis of the iron oxide by precipitation followed by coating with a polymer like dextran or other material. This type of synthetic route is utilized by Elmore, Phys. Rev. 54, 309 (1938) and Ohgushi et al., J. Mag. Res., 29, 599 (1978); both of which are incorporated herein by reference.

5   With proteins and dextrans, synthesis of the oxide in the presence of the polymer seems to effect a tight association between the polymer and the oxide. The synthesis of oxide first, followed by exposure to a protein or dextran yields a coated particle with the coating being held to the particle surface by relatively weak adsorption phenomena. However, if the oxide and adsorbed polymer can be manipulated, stored and injected in the presence of nonadsorbed polymer, the weakness of the association between oxide and polymer is not a problem. For example, when the particles of Sec-

10   tion 6.1.3. (uncoated) are diluted 1:1 into a neutral buffer containing 1% w/v human serum albumin (HSA), considerable protein will adsorb to the oxide surface. This approach to the synthesis of an albumin coated magnetic particle is a practical one for an imaging agent. The HSA coated particle (plus HSA in solution) can be injected into a patient and the HSA in solution mixes with HSA in serum. When particles are made by this approach the loosely associated HSA can be removed by treatments such as moderate temperature (50°C) or high salt (1M NaCl).

15   The coating methods are general and can be performed with a variety of physiologically acceptable proteins and carbohydrates, particularly those with molecular weights from about 5,000 to about 250,000 daltons. Other polymeric coatings include, but are not limited to, albumin/dextran composites, ficoll, dextrin, starch, glycogen and polyethylene glycol.

20   6.1.1. PREPARATION OF POLYSACCHARIDE-COATED PARTICLES

Polysaccharide-coated superparamagnetic iron oxide particles (about 10 to about 5000 angstroms in diameter) useful as MR contrast agents are prepared by a single-step process according to the procedure of Molday [U.S. Patent No. 4,452,773] incorporated herein by reference above. In a preferred embodiment, dextranized divalent (Fe^{2+}) and tri-

25   valent (Fe^{3+}) iron salts, e.g., $FeCl_2$ and $FeCl_3$, are precipitated from an aqueous solution containing a mixture of the iron salts and dextran (molecular weight of dextran can vary from 5,000 to 250,000 daltons) by the dropwise addition (to pH=10) of base ammonium hydroxide at 60-65°C, followed by centrifugation at 1500 x g for 15 minutes to remove the oversized particles which are subsequently discarded. The remaining particles are dialyzed against distilled water and can be concentrated by ultrafiltration. Any unbound dextran can be removed by gel filtration chromatography in a chlo-

30   ride/acetate buffer.
The ratio of Fe^{3+} to Fe^{2+} is preferentially maintained at about 2:1, but can be varied from about 0.5:1 to about 4.0:1 without substantial changes in product quality and efficiency as contrast agents.
Likewise, bases other than ammonium hydroxide (NH_4OH) can be used, but NH_4OH is preferred because the ammonium ion has a slight dispersing effect on iron oxides which increases the yield.

35   As mentioned above, various magnetically active metals notably Co, and Mn, may be substituted for Fe without any deleterious effect on the efficiency of the particles as contrast agents. Use of other polysaccharides such as starches, glycogen or dextrins is also contemplated.

6.1.2. PREPARATION OF PROTEIN-COATED PARTICLES

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Protein-coated superparamagnetic iron oxide particles are prepared by a single-step process similar to that of Molday [U.S. Patent 4,452,733]. The protein-coated particles can be prepared like the dextran coated wherein the iron salts (e.g., $FeCl_2$ and $FeCl_3$) and the protein are dissolved in water and the coated iron oxide particles are precipitated by the dropwise addition of base (NH_4OH) to pH=10. In an alternative embodiment the protein can be dissolved in the base

45   and an aqueous solution of the iron salts can be added dropwise to form a coated particle.
In either method, the oversized particles are subsequently collected by centrifugation at 1500 x g and the remaining particles are subjected to dialysis against distilled water followed by ultrafiltration. Any unbound protein can be removed by gel filtration chromatography in a chloride/acetate buffer.
As with the polysaccharide coated particles, both the coating composition and the Fe^{3+}/Fe^{2+} ratio (about 2/1) can

50   be varied from about 0.5:1 to about 4:1 without any deleterious effect on the efficiency of these particles as contrast agents.
As mentioned above, various magnetically active metals notably Co, and Mn, may be substituted for Fe without any deleterious effect on the efficiency of the particles as contrast agents.

55   6.1.3. PREPARATION OF UNCOATED PARTICLES

Uncoated superparamagnetic iron oxide particles are prepared by mixing an aqueous solution of ferric chloride ($FeCl_3$) with ferrous chloride ($FeCl_2$) in HCl and precipitating in 0.7 molar aqueous ammonia. The base precipitation offers a dual advantage in that the base reacts with the iron chlorides to form uncoated superparamagnetic iron oxide

particles. The precipitate (a gelatinous substance) is then collected by centrifugation or application of a magnetic field followed by decantation of the liquid phase.

The gel is then peptized to form a dispersoid by mixing with either 1 molar aqueous tetramethylammonium hydroxide (to form an alkaline dispersoid) or 2 molar aqueous perchloric acid (to form an acidic dispersoid) followed by cen-
5   trifugation and redispersion in water. Both of these dispersoids show remarkable stability and, being colloidal in nature, will not possess large solid particles. The counterions, either tetramethlylammonium hydroxide or perchlorate, are charged in basic or acidic media, respectively and, thus, prevent complex coagulation in solution; the particles (complexes of iron oxide/counterions) can be repeatedly precipitated and re-dispersed in solution and will retain this property.
10 In an alternative embodiment the particles can be collected by the application of an external magnetic field rather than centrifugation. The resultant magnetic cake is then peptized by the appropriate counterion.

The ratio of Fe^{3+}/Fe^{2+} is preferably maintained at about 2/1, but can be varied between about 0.5/1 and about 4/1. Decreasing the ratio will produce larger and increasing the ratio will produce smaller sized particles. Using the 2/1 ratio and 0.7M NH_4OH, the average particle size produced is about 1200 angstroms as measured by light scattering.
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6.2. USE OF THE PARTICLES AS MR IMAGING AGENTS

The magnetic materials described above can be used as contrast-enhancing agents for in vivo MR imaging. In one embodiment, the particles are dispersed in a suitable injection medium, such as distilled water or normal saline, or any
20 other physiologically acceptable carrier known in the art, to form a dispersoid which is introduced into the subject's vascular system by intravenous injection. The particles are then carried through the vascular system to the target organ where they are taken up.

When intravascularly administered, the particles will be preferentially taken up by organs which ordinarily function to cleanse the blood of impurities, notably the liver, spleen, and lymph nodes, and the other organs which tend to accu-
25 mulate such impurities, notably bone and neural tissue and to some extent, lung. In each of these organs and tissues, the uptake into the reticuloendothelial cells will occur by phagocytosis, wherein the particles enter the individual cells in membrane-bound vesicles; this permits a longer half-life in the cells, as such membrane-bound particles will not tend to clump or aggregate (aggregates are rapidly metabolized and cleared from the organ/tissue). Other uptake mechanisms are possible, e.g., pinocytosis. Also, it is possible that the other cells of the liver (hepatocytes) may absorb the
30 magnetic particles.

Because cancerous tumor cells can lack the ability of phagocytic uptake, the intravascularly administered particles can serve as valuable tools in the diagnosis of cancer in the above-mentioned organs, as tumors will be immediately distinguishable on any image obtained.

In a another embodiment, the particles are administered as dispersoids in a physiologically acceptable carrier,
35 such as distilled water, into the gastrointestinal tract, which includes the esophagus, stomach, large and small intestine, either orally, by intubation, or by enema, in a suitable medium. The particles are preferentially absorbed by the cells of the tract, especially those of the intestine and, like the intravascularly introduced particles, will exert an effect on T_2 of the organ or tissue. In this manner, cancers and other debilitating diseases of the digestive system such as ulcers can be diagnosed and affected areas pinpointed.

40 Regardless of the route, once administered, the particles distribute to and collect rapidly in the target organs, generally in 30-minutes to an hour.

In the organ, these superparamagnetic particles will alter the magnetic fields produced by the MR imager. These altered fields will exert an effect on the magnetic properties of the hydrogen nuclei (protons) in neighboring molecules; notably affected is the spin-spin relaxation time, T_2. This parameter is shortened which can result in image darkening.
45 Thus, the contrast is enhanced between areas which absorb the particles rapidly and those which absorb them slowly or not at all.

The particles are, however, ultimately biodegradeable and the iron can be utilized by the body for physiological requirements. The contrast effect will vary with the dose, being longer at higher doses, and also with the organ imaged. Particularly in the liver and spleen (which store iron for physiological use) the effect can be observed for 14 days or
50 more, (see Section 7.6), and, often, as long as 30 days.

The localization in these organs, which store iron for ultimate incorporation into hemoglobin, reveals that the iron oxide particles will ultimately serve as a source of metabolizable iron and, in fact, will be incorporated in the subjects hemoglobin. Thus, these materials can also be useful in the treatment of anemia.

The differences in parameter values are interpreted by computer and used to generate an image of the organ in
55 question. In the cases, as mentioned above, where uptake occurs by phagocytic processes (notably the liver, spleen, lymph nodes, and bone and neural tissue and to some extent, lung) such an image will clearly and distinctly differentiate between cancerous and healthy tissue, allowing for tumor location. In other organs and/or in the diagnosis of other diseases, modifications of the coating of these particles by the attachment of various functional groups will stimulate uptake by the organ or cell of choice. For example antibodies to a particular tumor cell (e.g. lung carcinoma) can be

attached to the surface of a coated particle, stimulating uptake by that organ if such a cell is present. In this way, the method can serve a diagnostic tool for many diseases.

6.3. PREPARATION OF SUPERPARAMAGNETIC FLUIDS

The superparamagnetic fluids useful as imaging agents in this invention ore preferably prepared in a three step process which comprises the steps of: formation of a superparamagnetic metal oxide; oxidation and dispersion of this oxide by sonication; and dialysis in buffer. This process yields stable biodegradable superparamagnetic metal oxides that owe their stability primarily to their anion retaining properties. The metal oxides may be uncoated or attached to organic coatings. Each of these steps is discussed separately below.

6.3.1. FORMATION OF SUPERPARAMAGNETC METAL OXIDE

Formation of the superparamagnetic metal oxide is accomplished by mixing the appropriate metal salts with a base. In a preferred embodiment, this is accomplished by mixing an aqueous solution or suspension of divalent and trivalent iron salts ($FeCl_2$/$FeCl_3$) with a base such as sodium hydroxide (NaOH). In addition, metals similar in structure to iron, such as Co and Mn, can be incorporated into the ultimate superparamagnetic fluid by replacing a portion, preferably 1/2 or less, of the divalent iron salt with a divalent salt of, that metal. The result is then a mixed metal oxide precipitate containing both ferrous and ferric oxides, as well as oxides of the divalent metal.

When iron salts are used the ratio of Fe^{3+}/Fe^{2+} can be varied from 1/4 to 4/1 and still produce usable product. Thus, a wide range of salt mixtures can be utilized.

Once the salts are mixed with the base, a superparamagnetic metal oxide precipitate is formed. The use of a high concentration of reactants and an abrupt change in pH favors the formation of small superparamagnetic metal oxides. Such oxides are preferred for use in the subsequent steps of this process.

6.3.2. DISPERSION AND OXIDATION

In the second process step, the superparamagnetic metal oxide prepared in 6.3.1. is dispersed and oxidized further by sonication. The sonication, which can be conducted at ambient or elevated temperatures (up to 100°C) serves a dual purpose: it serves to disperse any clusters of superparamagnetic particles (which increases the ultimate effects of the material on proton relaxation and, hence, enhances their effectiveness as MR contract agents) and, additionally, it serves to oxidize most, if not all, of ferrous oxide (Fe^{2+}) to ferric oxide (Fe^{3+}). The resultant material, remarkably, is a soluble superparamagnetic iron oxyhydroxide which forms a superparamagnetic fluid.

The sonication can be accomplished in any commercial apparatus including in a continuous flow sonicator or by a sonic probe. The former is especially useful when large volumes of materials are being handled and, for a continuous process, can be coupled with a heating and cooling apparatus to permit heating of the iron oxide prior to or after sonication (to increase the dispersion and oxidation of the oxides) and subsequent cooling of the sonicated mixture to facilitate collection.

6.3.3. DIALYSIS

The final step in the process is the transfer of the solution to an aqueous polycarboxylic buffer suitable for in vivo use. This transfer is accomplished by dialyzing the fluid against the buffer at neutral pH, generally at a pH of 6-9, preferably 6-8.3. These conditions result in a stable superparamagnetic fluid; under acidic conditions (below a pH of about 6) a significant amount of a chelate of the iron begins to form rather than the superparamagnetic iron oxide.

In the process, the fluid from 6.3.2. is centrifuged, to remove larger oxide aggregates, and the supernatant is dialyzed against the buffer. The preferred buffer contains a citrate salt, because of its suitability for in vivo use and its long history as an injectable agent, but in general buffers containing salts of any polycarboxylic acid, (such as tartrate, succinate) or maleate, allow for the formation of stable superparamagnetic fluids. The resultant fluids can then be autoclaved and stored until needed.

6.3.4. PREPARATION OF STABLE SUPERPARAMAGNETIC FLUIDS CONTAINING METAL OXIDES WITH ORGANIC COATINGS

Superparamagnetic fluids containing metal oxides to which organic coatings are attached can be prepared by modifications of the above procedure. Such organic coatings can be selected from a wide array of polymeric materials including, but not limited to, carbohydrates such as dextran (preferably having a molecular weight between 5,000 and 250,000 daltons), proteins (preferably having a molecular weight between 5,000 and 250,000 daltons) such as bovine or human serum albumen, polypeptides (preferably having molecular weights between 5,000 and 250,000 daltons)

such as polylysine and polyglutamates and organosilanes (preferably having a molecular weight between 5,000 and 250,000 daltons) such as N-2-aminoethyl-3-aminopropyltrimethoxysilane. Briefly, the attachment can be accomplished during either the first or the second steps.

When attachment is accomplished during the first step, the coating material is mixed with the salt solution prior to the supermagnetic metal oxide precipitation. The coating material is attached to the resultant precipitate, and remains attached during the subsequent steps. Any unbound coating agent is removed during dialysis (step 3). In a preferred embodiment, superparamagnetic fluids containing dextran-coated iron oxides can be formed in this manner.
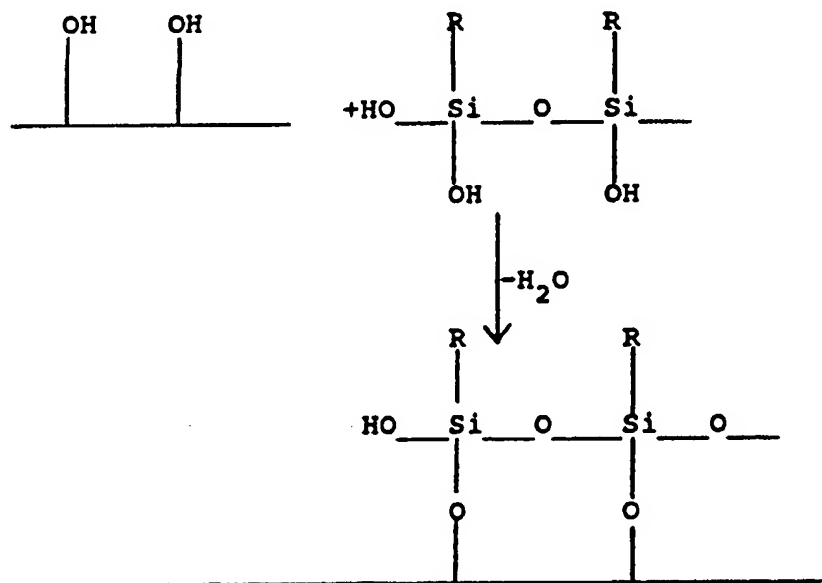
The attachment can also occur during the dispersion and oxidation second step by adding the coating agent prior to the sonication and subsequently sonicating the mixture to form the corresponding oxyhydroxide. Again, the unbound coating agents are removed by dialysis.

Superparamagnetic fluids containing silanized iron oxides are prepared in a similar manner. Initially, the iron oxides are subjected to sonication to form the oxyhydroxides. The organosilane is then added and the mixture is sonicated to disperse the materials. Finally, the silane is attached to the surface via a dehydration reaction. The polymerization of the silane may occur before or after the deposition on the oxyhydroxide surface.

In one embodiment, the silanization reaction occurs in two steps. First, a trimethoxysilane is placed in the sonicated mixture which condenses to form silane polymers:

$$R-Si(OCH3) \longrightarrow HO-\underset{\underset{OH}{|}}{\overset{\overset{R}{|}}{Si}}-O-\underset{\underset{OH}{|}}{\overset{\overset{R}{|}}{Si}}-O-$$
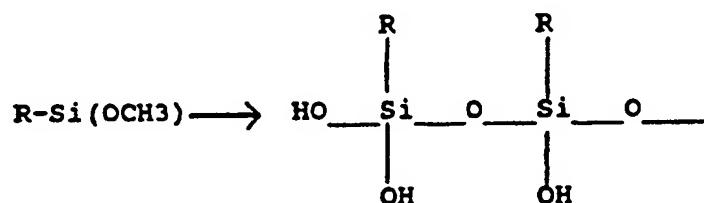
The mixture is then sonicated, after which these polymers associate with the metal oxide, presumably by forming a covalent bond with surface OH groups through dehydration:

$$\underset{|}{\overset{OH}{|}} \quad \underset{|}{\overset{OH}{|}}$$

$$+HO-\underset{\underset{OH}{|}}{\overset{\overset{R}{|}}{Si}}-O-\underset{\underset{OH}{|}}{\overset{\overset{R}{|}}{Si}}-$$

$$\downarrow -H_2O$$

$$HO-\underset{\underset{O}{|}}{\overset{\overset{R}{|}}{Si}}-O-\underset{\underset{O}{|}}{\overset{\overset{R}{|}}{Si}}-O-$$

Adsorption of silane polymers to the metal oxide is also possible.

An important aspect of this procedure is the method of dehydration used to effect the adsorptive or covalent binding of the silane polymer to the metal oxide. This association is accomplished by heating the silane polymer and metal oxide in the presence of a wetting agent miscible in both the organic solvent and water. Glycerol, with a boiling point of about 290°C., is a suitable wetting agent. Heating to about 105°C in the presence of glycerol serves two purposes. It insures the evaporation of water, the organic solvent (which may be e.g., methanol, ethanol, dioxane, acetone or other

moderately polar solvents) and any excess silane monomer. Moreover, the presence of glycerol prevents the aggregation or clumping and potential cross linking, of particles that is an inherent problem of other silanization techniques known in the art wherein dehydration is brought about by heating to dryness. Thus, few aggregates are formed. Any formed aggregates are removed by centrifugation and the unbound silane is removed by hydrolysis.

6.3.5. ADVANTAGES OF THE SUPERPARAMAGNETIC FLUID PREPARATION PROCESS

The process used to prepare the superparamagnetic fluids of this invention is uniquely suited for preparing magnetic fluids suitable for in vivo use. Specifically, the following advantages are noted:

1. At no time is the material dried, nor is it precipitated after the intial formation of the superparamagentic oxides. Such operations bring particles into close proximity with each other resulting in clustering and aggregation, which adversely affects their utility as MR contrast agents. Further, at no time are the metal oxides removed from superparamagnetic fluid by precipitation or filtration; in fact, they cannot be so removed. In dilute concentrations, the metal oxides will pass through a $0.22 \cdot 10^{-6}$ m (0.22 micron) filter.

2. Because the material is never precipitated (after the initial formation of the iron oxide), acids or bases are not needed to resolubilize the iron oxide. Use of acids tends to dissolve iron oxides, yielding ferric ion which is toxic and must be removed prior to in vivo use. Strong bases are also poorly suited for use in the preparation of pharmaceutical solutions of superparamagnetic fluids. Strong bases can hydrolyze biological molecules attached to iron oxides such as proteins or polysaccharides. Amine-containing strong bases can react with polysaccharides in the well known Malliard reaction.

3. Changes in solvents, such as to citrate buffer, are accomplished by dialysis. Many other methods (such as that described in U.S. Patent 4,001,288) of iron oxide preparation require removal of iron oxides from solution to accomplish changes in solvent, often using acid or base to resolubilize the precipitate.

4. The attachment of coating materials to the particles during the preparation permits a wide array of biologically active molecules such as antibodies, antigens, serum proteins or other materials to be attached. The attached biologically active molecule can serve to direct the superparamagnetic agent in vivo, as described in section 6.5.

6.4. CHARACTERSTICS OF SUPERPARAMAGNETIC FLUIDS

6.4.1. MAGNETIC PROPERTIES

The fluids produced by the methods described in section 6.3. are characterized by a high magnetic moment in a high magnetic field (generally, about 5 to about 90 EMU/gm of metal oxide) and a negligible magnetic moment in the absence of an applied field (i.e., a magnetic squareness of less than 0.1). Such behavior is characteristic of superparamagnetic particles.

FIG. 4 shows magnetic hysteresis loops for typical paramagnetic, superparamagnetic and ferromagnetic iron oxides. Magnetization was measured in a vibrating sample magnetometer with fields up to 6,000 Gauss, 25°C. At high magnetic fields, the superparamagnetic fluid of the invention is nearly as magnetic as ferromagnetic iron oxide and far more magnetic than the paramagnetic ferric oxyhydroxide. Also, the solutions of the invention are superparamagnetic rather than ferromagnetic, losing virtually all of their magnetic moment in the absence of an applied magnetic field. In fact, the superparamagnetic solutions of this invention are characterized by a saturation magnetization of 30 EMU/gm or greater, with the loss of more than 90% of this magnetism in the absence of a magnetic field.

6.4.2. RETENTION OF CITRATE AND STABILITY OF SUPERPARAMAGNETIC FLUIDS

The retention of citrate (from aqueous sodium, potassium, or ammonium citrate buffer) can be used to distinguish the superparamagnetic fluids of this invention from other iron oxides. Studies of citrate binding capacity of commercially available forms of iron oxides and ferric oxyhydroxides reveals that the iron oxides in the superparamagnetic fluids of this invention are capable of retaining nearly as much citrate as the paramagnetic (ionic) ferric oxyhydroxide, while gamma ferric oxide and magnetite cannot retain significant amounts of citrate. The inability of iron oxides to retain citrate, coupled with the ability of ferric oxyhydroxide to do so, strongly suggest that citrate does not adsorb to the surfaces of iron oxides prepared according to the method of this invention (see Section 6.3) through the usual chemical adsorption mechanism. The retention of anions like citrate by the superparamagnetic iron oxides of the invention indicates these materials have an ionic character similar to the ferric oxyhydroxides.

The stability of fluids of the invention is shown in FIG. 5, where a superparamagnetic fluid made according to the procedure described in section 6.3. was subjected to autoclaving with and without citrate. Addition of 50 mM citrate stabilized a solution of 1.26 M iron oxide, preventing the gellation of the material.

The stability of the oxyhydroxide solutions of iron (i.e., the superparamagnetic fluids) is related to the exchange of hydroxide for citrate ion. Both paramagnetic and superparamagnetic oxides retain citrate in a similar fashion:

$$3 \text{ FeO:OH} + \text{Citrate}^{3-} \rightarrow (\text{FeO})_3-\text{Citrate} + 3 \text{ OH}^-$$
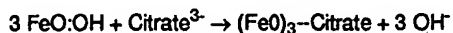
Instead of trying to block the Van der Waals forces between neutral crystals with polymers, by attaching surfactants, or forming complexes, the general approach used by others in forming ferrofluids, the invention's superparamagnetic fluids are stabilized due to the ionic character of the iron oxide and the choice of appropriate anions.

The stable solutions of this invention comprise a metal concentration ranging from 0.05 to 5 molar and a citrate ion concentration of 0.001 to 0.1 moles of citrate/mole preferably 0.01 to 0.1 moles of citrate/mole of iron at a pH ranging from about 6 to about 10. As the concentration of iron in the solution is increased, the ratio of citrate/iron must also increase to yield the stability. Thus, they are compatible with physiological conditions.

The superparamagnetic fluids of the invention owe their stability in solution, not to their coating with polymers or surfactants, but to the existence of a cationic character of the iron oxide and its stabilization with anions such as citrate. In general, polymeric coatings, though they help stabilize iron oxides, are not sufficient to protect them against the rigors of autoclaving. In contrast, superparamagnetic fluids made according to the invention can be made omitting the polymer altogether and are highly stable.

6.4.3. EFFECTIVENESS AS MR CONTRAST AGENTS

In evaluating magnetic materials as MR contrast agents, the ability of materials to shorten proton relaxation time can be more important than bulk magnetic properties such as magnetization. Since MR imaging works by determining the rates of two types of proton relaxations in various tissues and, by using variations in those relaxation rates, develops an image, the differences in proton relaxation times between the tissues must be sufficiently great to obtain a good quality image. As stated supra, MR contrast agents work by shortening proton relaxation time and, thus, increases the contrast and overall image quality. Two relaxation parameters termed spin-spin relaxation time (T_1) and spin-lattice relaxation time (T_2) are used in the generation of the MR image.

In experiments evaluating the effect of these materials as contrast agents, it was found that the superparamagnetic fluids have a much greater effect on both T_1 and T_2 than any commercially available iron compounds including chelated ferric ion, paramagnetic ferric oxyhydroxides, gamma ferric oxides, and superparamagnetic iron oxide clusters (Example 7.15). In fact, the material of the invention is remarkable in its ability to shorten proton relaxation. The materials prepared according to the invention are more potent enhancers of proton relaxation than either ferromagnetic materials or paramagnetic ferric oxyhydroxide. In addition, the highly dispersed state of the materials of the invention produces higher relaxivities than those characteristic of clustered materials (See 7.12 and Table III). The process, thus, yields superparamagnetic solutions optimized for their effects on proton relaxation time.

The high relaxivity (see Table III) of the materials of the invention is important to their pharmaceutical use as MR contrast agents, because it results in large effects on the MR image with small dozes of iron. For example, superparamagnetic iron oxides made according to the invention can profoundly improve liver imaging at doses of 1 mg of iron per kilogram of rat, while the LD50 for the rat is greater that 250 mg of iron per kilogram.

6.5. BIODEGRADABILITY

Both the superparamagnetic particles in the dispersoids and the metal oxides in the superparamagnetic fluids of the invention have been found to be biodegradable when administered in vivo (see Examples 7.6 and 7.15). In fact, iron, the predominant species in the dispersoids and fluids accumulate in the liver, where it is eventually catabolized and incorporated into the subject's hemoglobin. Thus, the dispersoids and fluids can be used in the treatment of anemia, and indeed, the fluids have been shown to be as effective as Imferon (a commercially used preparation for treatment of anemia in humans) in the restoration of normal hematocrit levels in anemic rats.

6.6. DIRECTABILTY

Both the superparamagnetic particles in the dispersoids and the metal oxides in the superparamagnetic fluids of the invention can be coated with various coating agents as described supra. The use of such coatings permits the attachment of various biological molecules to the imaging agents to permit targeting of various organs. For example, antibodies can be bound by a variety of methods including diazotization and attachment through a glutaraldehyde or carbodiimide coupling moiety (Examples of these coupling methods can be found in U.S. Patent No. 4,628,037). Use of

methods such as these permits maximum flexibility, as an antibody-directed superparamagnetic metal oxide can bind to a specific type of cell or tissue. This can permit an image to be generated which differentiates between the target and the surrounding tissue.

In addition to antibodies, other biological molecules which affect directability can also be attached to the particles as the particular application dictates. Some possible applications are listed below:

Antibodies	Application
1. Anti-heart myosin	Imaging infarcted area of heart
2. Anti-fibrin	Image clot
3. Anti-T-cells	Lymphoma
4. Anti-CEA	Colonic tumor imaging
5. Anti-melanoma antibodies	Melanoma imaging
6. Anti-ovarian cancer antibodies	Ovarian cancer imaging
7. IgG	Fc receptor delineation

Carbohydrates

1. Bacterial lipopolysaccharides
2. Cellulose
3. Mucopolysaccharides
4. Starch
5. Modification of carbohydrate after synthesis, e.g., dextran coating made positively or negatively charged, diethyl-amino (DEAE) cellulose or carboxymethyl (CM) dextran.

Hormones	Application
1. Insulin	Insulin receptor status as in maturity onset diabetes
2. Thyroid stimulating hormone	Thyroid disease
3. Acetylcholine (or analogs)	Delineation of neural receptors
4. Serum low density lipoprotein	Delineation of familial hyper cholesterolemia
5. Hormone analogs including drugs	Delineation of endocrine system and receptors
6. Serum transferrin	Transferrin receptors delineation

6.7 METHOD FOR EXTENDING THE SERUM LIFETIME OF AN MR IMAGE CONTRAST AGENT

To extend the lifetime of an MR imaging agent in the serum of a subject, if desired, it is neccessary to prevent its absorption by the reticuloendothelial system (RES). It has been found that this can be accomplished by introducing to the subject a blocking agent which effectively competes with the imaging agent for binding the RES receptors responsible for removing the MR contrast agent from the bloodstream. There are a number of phagocytic receptors which function independently of each other. As a result, no single material is equally effective at blocking all the RES receptors and any blocking agent must be specific for the imaging agent. (See Davis et al. in "Polymeric Nanoparticles and Micro-spheres", Gurot, P. and Covreur, P., eds, (CRC Press, 1986) p. 180).

In the procedure, the subject is given a dose of paramagnetic iron oxide either prior to or along with the administration of the imaging agent. For the best results, the paramagnetic iron oxide should be as similar to the agent as practical

especially in particle size and coating. After a short time interval, generally 15-20 minutes during which time the paramagnetic material circulates in the bloodstream and binds to the RES receptors, the imaging agent is administered. By proper choice of the paramagnetic dosage, the lifetime of the imaging agent in the serum is greatly enhanced.

An excellent blocking agent for a superparmagnetic MR agent is a paramagnetic form of the same material. This is
5 because the effectiveness of a blocking agent depends on whether a competition for receptors results; cell surface receptors bind materials in circulation prior to internalization. This internalization is termed pinocytosis (removal of liquids) or phagocytosis (removal of particles). If competition is created, which blocks removal of the superparamagnetic MR contrast agent, the removal of the contrast agent will be hindered. Because the RES receptors are specific and will bind substances of only one particular size or shape, this competition is best observed among materials which are
10 physically similar. Since a paramagnetic particle can differ from a superparamagnetic particle only in its core magnetic properties and rather than its surface chemistry, a high degree of competition is inevitable and, thus, the paramagnetic material is a highly efficient blocking agent.

In a preferred embodiment, dextran-coated paramagnetic iron oxide is used as a blocking agent for dextran coated superparamagnetic iron oxide. This material is ideal as a blocking agent for dextran-coated superparamagnetic iron
15 oxide contrast agents for the reasons below:

1. It is virtually undectectable by MR.

2. It can be made by the process used for superparamagnetic materials, but without the use of divalent iron, (which
20 is required for the superparamagnetic product). The MR contrast agent and blocking agent are identical except for the fine structure of the iron oxide which determines magnetism and detectability by MR. From the point of view of the cell surface receptor governing removal from circulation the superparmagnetic imaging agent and blocking agent are identical.

25 3. It is non-toxic in humans and has an established therapeutic use in the treatment of anemia. In fact, therapeutically approved paramagnetic dextran (Imferon) can be used as a blocking agent for the superparmagnetic MR contrast agents of the invention.

The extension of serum lifetime is of particular importance when MR measurements are used to confirm blood cir-
30 culation (or lack thereof). In such measurements, the contrast agent is introduced parenterally and permitted to circulate. By measurement of T_1 and T_2, presence or absence of blood circulation can be determined. Such a procedure can be a valuable tool in the diagnosis of blood circulation disorders, and can be used to detect the flow of blood into areas where it is normally excluded, such as in strokes.

35 7. EXAMPLES

7.1. PREPARATION OF DEXTRAN-COATED PARTICLES

To a solution of 500 mls of 0.28M $FeCl_3$, 0.16M $FeCl_2$ and 12.5% w/v dextran, (molecular weight 71,000 daltons
40 from Sigma Chemical Company, Cat. #D1390) is added 500 mls 7.5% NH_4OH over a 2 minute period. A black, magnetic solid forms comprised of large and small particles. The material is stirred for 5 minutes and then heated for 30 minutes at 70°C. The solution is centrifuged for 1500 x g for 15 minutes to remove large particles, and the small particles are dialyzed against 10 gallons of H_2O for three days, changing the water each day.

The resultant particles exhibit a diameter of about 1400 angstroms as measured by light scattering.
45
7.2. Preparation of Particles Coated with Bovine Serum Albumin

To a solution of 80 mls of 0.5% bovine serum albumin (BSA), 0.27M $FeCl_3$, and 0.16M $FeCl_2$, is added 80 mls of 7.5% NH_4OH. A black, magnetic solid forms comprised of particles. The mixture is allowed to stand for 5 minutes and
50 then centrifuged at 1,500 x g for 15 minutes to remove larger particles. The pellet is discarded and the supernatant placed in a dialysis bag and dialyzed against 3 changes of 10 gallons of distilled water. Larger particles are again removed by centrifugation as above and discarded. Particles are then concentrated by ultrafiltration using an XM-50 membrane and a stirred cell filtration device from Amicon Corporation, Lexington, MA.

The resultant particles exhibit a diameter of about 1730 angstroms as measured by light scattering.
55
7.3. PREPARATION OF UNCOATED PARTICLES

One hundred milliliters of solution of 0.8M $FeCl_3$, O.4M $FeCl_2$ and 0.4M HCl is added dropwise to 1000 ml of 2.4% NH_4OH and mixed for 5 minutes. A black, magnetic solid forms comprised of easily visible particles. For particles to be

visible, they must be larger than the wavelength of scattered light which is about 500 nm (0.5 microns). The particles are isolated by attracting them to a permanent magnet on the outside of the reaction vessel and the solution decanted. To the magnetic cake is added 55 mls of 50% triethylamine in water. Smaller particles are created. The mixture is dialyzed overnight against water which causes the large particles to reappear. Just enough triethylamine is then added to again create the smaller particles resulting from the addition of triethylamine. The particles are then filtered through a 0.2 micron filter indicating the final material is below this size.

7.4. USE OF PARTICLES IN LIVER TUMOR VISUALIZATION

The effect of the dextran-coated particles of Section 7.1. on the image of a rat liver tumor is demonstrated in FIG. 2, which presents reproductions of five images obtained on a Technicare MR imager. The images in FIGS. 2A and 2B were obtained prior to the introduction of the imaging agent using different imager settings, in neither case can the tumor be clearly seen; FIGS. 2C and 2D are images of the same rat liver and were obtained after a single 0.5mg/kg dose of the Section 6.1. dextran-coated particle by intravenous injection through the tail vein, the tumor is easily seen and the overall size and shape can be gauged; in FIG. 2E the tumor is marked by cross-hairs to aid in visualization.

7.5. COMPARATIVE EFFECT OF SUPERPARAMAGNETIC PARTICLES AND FERROMAGNETIC PARTICLES ON T2

FIG. 1 compares the T_2 of agar gel in the presence of dextran-coated particles (produced in Example 7.1.) and the ferromagnetic particle Pf-2228 (Pfizer). The relaxation times in the presence of varying concentrations of each particle were determined on an IBM PC-20 NMR spectrometer at 0.47 Tesla (4700 Gauss). It can clearly be seen that the superparamagnetic particle produces a much greater effect on T_2 than the ferromagnetic particle. Given the fact that superparamagnetic materials are much less magnetic than ferromagnetic materials, this result is quite surprising.

7.6. BIODEGRADABILITY OF DEXTRAN-COATED PARTICLES

A dispersion of uncoated superparamagnetic iron oxide particles in water was intravenously injected into Sprague-Dawley rats at dosages of 20, 37 and 243 micromoles Fe/kg of body weight. Periodically, the rats were sacrificed, and T_2 of the liver tissue was measured on an IBM PC-20 NMR Spectrometer. The results are presented in FIG. 3.

The data demonstrate that T_2 undergoes a marked drop rapidly after the injection, and then begins to recover slowly, presumably as the iron is metabolized. However, the effects are still detectable even two weeks after administration. Also, the effects are more marked with the higher dosages. Thus the particles have an extended lifetime in these organs. Since the liver and spleen are the major organs which store iron for incorporation into hemoglobin, it is postulated that these materials are incorporated into the hemoglobin of the rat.

7.7. BIODISTRIBUTION OF BSA-COATED PARTICLES

Six Sprague-Dawley rats of about 200 gm each were injected intravenously with 0.4 mg of the BSA-coated particle (produced in Section 7.2.) in distilled water. Two rats each were sacrificed at 90 minutes, 24 hours, and 7 days after injection and the relaxation times (T_1 and T_2) of various organs were measured on an IBM PC-20 NMR Spectrometer. The following results were obtained:

TABLE I

DISTRIBUTION OF BSA-COATED PARTICLE IN RAT ORGANS AND TISSUES					
Time After Injection		Relaxation Times (msec)			
		Liver	Spleen	Lung	Blood
Control	T_1	0.279	0.544	0.688	0.786
$N^1 = 6$	T_2	32	48.3	57	158
90 min	T_1	0.232	0.396	0.656	0.901
N = 2	T_2	20	22	56	136
24 hours	T_1	0.279	0.494	0.735	1.084
N = 2	T_2	22	44	68	155
7 days	T_1	0.268	0.572	0.712	0.972
N = 2	T_2	31	49	68	162

[1] N is the number of rats examined.

The data suggest that both the blood and the lung rapidly clear the magnetic material exhibiting nearly no effect on the relaxation times 90 minutes after the injection. The spleen demonstrates a moderately rapid recovery, exhibiting a substantial reduction in both T_1 and T_2 90 minutes after the injection, but nearly no residual effect after 24 hours. The liver exhibits two different recovery rates. T_1 attains its original value after 24 hours, while T_2 remains substantially reduced after 24 hours and exhibits recovery after 7 days.

7.8. COMPARATIVE BIODISTRIBUTION OF UNCOATED AND DEXTRANIZED PARTICLES

In this experimental series, the biodistribution of three uncoated and four dextran-coated particles was examined. The uncoated agents were produced according to the procedure of Section 7.3., the dextran-coated particles were produced according to the procedure of Section 7.1. except that the molecular weight of the dextran used for the coating was varied (see TABLE II). Prior to each experiment, the contrast agents were dialyzed against distilled water and subsequently injected into separate groups of Sprague-Dawley rats in a distilled water carrier. The rats were periodically sacrificed and the relaxation times of the liver, spleen, and lung were determined on an IBM PC-20 NMR Spectrometer. Preprogrammed inversion recovery and Carr, Purcell, Meiboom, Gill pulse sequences were used to determine T_1 and T_2, respectively.

The results were as follows:

TABLE II

Complex	Coating	Dose	Time After Dose		Relaxation Times (msec.)		
					Liver	Spleen	Lung
Control	–	None	–	T_1	0.27	0.54	0.717
				T_2	32	48	64
AMI-12	None	24.2 μmoles/ kg	2.5 hr.	T_1	0.222	0.420	0.626
				T_2	22.7	26.0	45.8

Complex	Coating	Dose	Time After Dose		Relaxation Times (msec.)		
					Liver	Spleen	Lung
			18 hr.	T_1	0.254	0.532	0.752
				T_2	29.6	42.9	68.2
			1 wk.	T_1	0.239	0.528	0.730
				T_2	31.6	43.8	72.0
			2 wk.	T_1	0.240	0.462	0.702
				T_2	29.4	35.5	79.5
AMI-13	None	24.6 μmoles/ kg	2.5 hr.	T_1	0.221	0.424	0.672
				T_2	16.9	28.0	65.2
			18 hr.	T_1	0.218	0.386	0.802
				T_2	18.8	29.0	80.8
			1 wk.	T_1	0.236	0.443	1.753
				T_2	26.0	38.5	80.4
			2 wk.	T_1	0.236	0.493	0.722
				T_2	28.2	43.8	80.8
AMI-14	None	25.4 μmoles/ kg	2 hr.	T_1	0.238	0.470	0.706
				T_2	20.8	31.8	72.4
			18 hr.	T_1	0.238	0.436	0.750
				T_2	20.4	34.7	69.6

Complex	Coating	Dose	Time After Dose		Relaxation Times (msec.)		
					Liver	Spleen	Lung
			1 wk.	T_1	0.216	0.522	0.755
				T_2	26.7	41.7	80.4
			2 wk.	T_1	0.227	0.452	0.698
				T_2	24.8	43.6	78.7
AMI-15	Dex-tran 9,000	36.8 μmoles/ kg	4 hr.	T_1	0.238	0.300	0.672
				T_2	17.8	19.4	56.4
			24 hr.	T_1	0.253	0.387	0.740
				T_2	21.1	26.4	73.2
			1 wk.	T_1	0.219	0.485	0.766
				T_2	25.6	36.7	78.1
			2 wk.	T_1	0.258	0.523	0.718
				T_2	28.7	39.1	69.9
AMI-16	Dex-tran 17,900	32.4 μmoles/ kg	4 hr.	T_1	0.248	0.302	0.678
				T_2	18.8	16.5	56.2
			24 hr.	T_1	0.238	0.384	0.703
				T_2	19.9	24.9	71.6
			1 wk.	T_1	0.197	0.470	0.725
				T_2	25.3	37.1	74.6

Complex	Coating	Dose	Time After Dose		Relaxation Times (msec.)		
					Liver	Spleen	Lung
			2 wk.	T_1	0.258	0.525	0.731
				T_2	28.9	44.8	73.3
AMI-17	Dextran 35,600	33.1 µmoles/ kg	4 hr.	T_1	0.244	0.318	0.674
				T_2	16.0	17.4	54.4
			24 hr.	T_1	0.247	0.388	0.690
				T_2	20.2	22.9	76.4
			1 wk.	T_1	0.214	0.500	0.696
				T_2	24.3	44.0	76.0
			2 wk.	T_1	0.244	0.562	0.726
				T_2	28.6	48.6	70.6
AMI-18	Dextran 249,000	39.2 µmoles/ kg	4 hr.	T_1	0.228	0.237	0.526
				T_2	20.0	17.7	58.6
			24 hr.	T_1	0.238	0.354	0.654
				T_2	21.0	22.0	68.2
			1 wk.	T_1	0.235	0.492	0.645
				T_2	31.4	36.1	71.3
			2 wk.	T_1	0.240	0.52	0.748
				T_2	31.0	39.8	71.3

As before, the data suggest that the contrast agents are rapidly cleared from the lung, and are longer lived in the spleen and the liver. Additionally, it can be seen that the dextran-coated particles are cleared less rapidly than the uncoated ones, exerting a significant effect on the T_2 values of the liver and spleen for about one week.

7.9. PREPARATION OF SUPERPARAMAGNETIC FLUIDS CONTAINING UNCOATED METAL OXIDE

7.9.1 PREPARATION OF SUPERPARAMAGNETIC IRON OXIDE

A solution of 0.25 M ferrous chloride and 0.5M ferric chloride (600 ml) was poured into a solution of 5 M NaOH (600 ml). A black magnetic oxide precipatate was formed. This precipitate was washed repeatedly by base and decanted until a pH of about 9 was achieved.

7.9.2 DISPERSON AND OXIDATION

In a beaker, 400 ml of magnetic oxide (about 15 grams) from Section 7.9.1 and 25 ml of glacial acetic acid were mixed. A sonic probe was placed in the beaker and the solution was sonicated at high intensity for 2 minutes. The sonic probe was then removed and the solution centrifuged at 1,000 X g for 20 minutes. The pellet was discarded and the supernatant liquid was retained.

7.9.3 TRANSFER TO CITRATE BUFFER

The supernatant, from Section 7.9.2, was dialyzed against ammonium citrate buffer by use of a hollow fiber dia-lyzer/concentrator, model DC 2 (AMICON Corp. Danvers, MA). The ammonium citrate buffer is 10 mM citrate, adjusted to pH 8.2 with NH_4OH. The result is an autoclavable, homogeneous superparamagnetic fluid.

7.10. PREPARATION OF AN AQUEOUS, STABLE SUPERPARAMAGNETIC FLUID CONTAINING METAL OXIDE WITH DEXTRAN ATTACHED

7.10.1 SYNTHESIS OF IRON OXIDE

Five liters of a solution containing 755 g $FeCl_3$ $6H_2O$ and 320 g $FeCl_2$ $4H_2O$ was added slowly to 5 liters of 16% NH_4OH containing 2500 gm dextran (MW=10-15,000). The iron salt solution was added over 5 minutes during which time the base was vigorously stirred during addition.

A black magnetic slurry was formed.

7.10.2. DISPERSION, OXIDATION AND HEATING

The 10 liters of slurry (from Section 7.10.1) was pumped through a continuous flow sonicator connected to a 100°C heating coil and cooling coil apparatus as indicated in FIG. 6. The pumping rate was about 0.4 liters per minute and pumping was continued for about 30 minutes. The resultant solution was subsequently centrifuged and the precipitated pellet was discarded.

7.10.3. REMOVAL OF UNREACTED DEXTRAN, TRANSFER TO CITRATE BUFFER AND STERILIZATION

The supernatant (from Section 7.10.3) was diluted to a total volume of 20 liters with deionized, sterile water and the resultant solution was dialyzed as in Example 7.9, except that a larger dialyzer concentrator, the DC 10, was used. The dialyzer cartridge had a 100,000 dalton molecular weight cutoff, permitting removal of dextran. Ultrafiltration was accomplished in a noncontinuous fashion, reducing the volume from 20 to 5 liters and adding 16 liter volumes of solu-tion. Five volumes of 16 liters of deionized, distilled water were added.

Sodium citrate was then added as 1M citrate buffer stock and the solution was dialyzed as in Example 7.9. The resultant citrate was adjusted to pH 6.5 with NaOH before autoclaving. The citrate to iron ratio was between 0.01 and 0.1 citrate/Fe in the final solution. (For example, for an iron concentration of 1.26M, 0.04 M citrate was present. The magnetic fluid was bottled and autoclaved (121°C, 30 minutes). The result is a sterile homogenous magnetic fluid as shown in FIG. 5.

7.11. PREPARATION OF AN AQUEOUS STABLE SUPERPARAMAGNETIC FLUID CONTAINING METAL OXIDE WITH SILANE ATTACHED

7.11.1. PREPARATION OF IRON OXIDE

A solution of 0.25 M ferrous chloride and 0.5M ferric chloride (600 ml) was poured into a solution of 5 M NaOH (600 ml). A black magnetic oxide precipitate formed which was repeatedly washed by base and decanted until a pH of about 9 was achieved.

7.11.2. DISPERSION, OXIDATION AND SILANIZATION

In a beaker 400 ml of magnetic oxide (from Section 7.11.1., about 15 grams) and 25 ml of glacial acetic acid were mixed. A sonic probe was placed in the beaker and the solution was sonicated at high intensity for 2 minutes. The sonic probe was then removed and 30 ml of N-2-aminoethyl-3-aminopropyltrimethoxysilane was added. The resultant mixture was then sonicated as before. The magnetic solution was subsequently added to 200 ml of glycerol at 50°C. The temperature was raised to 105°C and the water was evaporated.

Due to the use of sonication, the material made in Example 3 is far smaller than that described in United States Patent No. 4,554,088. Due to its small size, it cannot be manipulated with hand held magnets. The glycerol dehydration step is from US Patent 4,554,088.

7.11.3. REMOVAL OF UNREACTED SILANE AND TRANSFER TO CITRATE BUFFER

The glycerol slurry, from Section 7.11.2, was added to about 800 ml of water. Large aggregates of magnetic particles were removed by centrifuging the slurry at 1,000 X g for 20 minutes. The supernatant was then dialyzed against citrate buffer in a hollow fiber dialysis device as in Example 6.9.

7.12. EFFECT OF THE SUPERPARAMAGNETIC FLUID ON PROTON RELAXATION TIME

The effects of materials on an in vivo MR image can be evaluated through the use of a magnetic resonance spectrometer. In this study, an IBM-PC 20 instrument which measures T_1 and T_2 at 25°C, 0.47 Tesla and 20 MHz was used. Enhancement of proton relaxation can be quantified by taking the slope of a plot of $1/T$, the reciprocal of the relaxation time, versus the concentration of contrast agent. The plot is generally linear, with the slope being termed the relaxivity and denoted R1 or R2. Relaxivity has units of M^{-1} sec^{-1}. Higher relaxivity values indicate that material is more potent per mole of iron at decreasing relaxation times of protons and, thus, is a more potent contrast again. Relaxivities for a number of different forms of magnetic materials were determined. The following materials were examined:

Superparamagnetic fluid of the invention: A dispersed fluid containing single superparamagnetic crystals of iron oxide prepared as described in Example 7.10. The magnetization curve of this material is presented in FIG. 4.

Fe_2O_3: A ferromagnetic gamma ferric oxide used for data recording. This material was obtained from Pfizer Corp., Minerals, Pigments and Metals Division, catalogue #2228.

Cluster: A silanized cluster of superparamagnetic iron oxide with tens to hundreds of crystals packed into micron-sized particles. This material was made according to U.S. Patent No. 4,554,088.

FeO:OH: a paramagnetic, ferric oxyhydroxide used in the treatment of anemia. It was obtained from Fisons Corporation and is sold under the trade names of Proferdex (Fisons corporation) or Imferon (Merrill Dow Inc.)

Fe^{3+}/DTPA: a soluble complex of ferric ion and diethylenetriaminepentaacetic acid (DTPA). (The data for this material is from Lauffer et al, J. Comp. Assist. Tomog. 9(3), 431 (1985)).

The results were as follows:

TABLE III

EFFECT OF DIFFERENT FORMS OF IRON ON PROTON RELAXATION TIME		
Material	R1 (M^{-1} X sec^{-1})	R2 (M^{-1} X sec^{-1})
superparamagnetic fluid	$4 \times 10^{+4}$	$1.6 \times 10^{+5}$
gamma Fe_2O_3	100	$7.2 \times 10^{+3}$
FeO:OH	0	60
Cluster	$2 \times 10^{+3}$	$3 \times 10^{+4}$
Fe^{3+}/DTPA	$0.73 \times 10^{+3}$	$0.85 \times 10^{+4}$

Briefly, as the high values of R1 and R2 indicate, the material of the invention is remarkable in its ability to shorten proton relaxation times. For comparison, the value of R2 for ferromagnetic dextran magnetite is $1.7 \times 10^{+4}$ M^{-1} sec^{-1} [Ohgushi et al., J. Mag Res. 29, 599 (1978)]. This is the highest literature value for R2 of which the authors are aware. The materials prepared according to the invention are more potent enhancers of proton relaxation time than either ferromagnetic materials or paramagnetic ferric oxyhydroxides.

Additionally, well dispersed materials, such as those of the invention, have higher relaxivities than clustered materials. Thus, the process of the invention yields superparamagnetic solutions optimized for their effects on proton relaxation.

7.13. BULK MAGNETIC PROPERTIES OF SUPERPARAMAGNETIC FLUIDS

Magnetic hysteresis loops were obtained for the samples of the invention superparamagnetic fluid, gamma Fe_2O_3 (ferromagnetic), and FeO:OH (paramagnetic) examined in Example 7.12, using a commercial vibrating sample magnetometer with fields up to 6000 Gauss, 25°C. The results are presented in FIG. 4.

Briefly, at high magnetic fields, the superparamagnetic fluid of the invention is nearly as magnetic as ferromagnetic iron oxide and far more magnetic than the paramagnetic ferric oxyhydroxide, showing a high magnetic saturation. The fluids of the invention are superparamagnetic rather than ferromagnetic, losing virtually all of their magnetic moment in the absence of an applied magnetic field.

7.14. RETENTION OF CITRATE

The retention of ^{14}C citrate upon dialysis can be used to distinguish various forms of iron oxide as shown in Table IV. All iron oxides were initially dialyzed against a buffer of 1 mM Tris-Cl, pH 8 before use. Equilibrium dialysis was then performed to determine fraction of citrate retained. The concentrations of iron and citrate were 17.8 and 2.6 mM, respectively. The superparamagnetic fluids of the invention retain amounts of citrate similar to commercially available ferric oxyhydroxides indicating that the iron in both preparations is in a similar chemical form. Commercially available forms of iron oxide, such as gamma Fe_2O_3 or Fe_3O_4, do not retain significant amounts of citrate (the gamma Fe_2O_3 was the same as that used in Examples 7.12 and 7.13 while the Fe_3O_4 was purchased from Fisher Scientific Inc). The inability of these commercially available iron oxides to retain citrate, coupled with the ability of ferric oxyhydroxide to do so, strongly suggests that citrate does not absorb to iron oxide surfaces through the usual chemical adsorption mechanism. The retention of citrate by the superparamagnetic iron oxides of the invention indicates these materials have an ionic character similar to the ferric oxyhydroxides.

TABLE IV

RETENTION OF CITRATE BY SOLUTIONS WITH DIFFERENT IRON OXIDES	
Material	Citrate Retained per Iron (mole/mole)
FeO:OH	0.026
Invention	0.019
gamma Fe_2O_3	0.0028
Fe_3O_4	0.0018

7.15. STABILITY OF SUPERPARAMAGNETIC FLUIDS

Superparamagnetic fluids made according to example 7.9 were subjected to autoclaving with various concentrations of citrate. At iron concentration of 1.26M, various concentrations of ammonium citrate, pH8 were added, and the resulting solutions heated 1 hour at 121°C. The results are presented in FIG. 5. The 6 vials of Fig. 5B contained, as shown, citrate concentrations of 100, 50, 25, 15, 10 and 5 mM citrate, respectively. The vials were upright during autoclaving but were placed horizontally for the photograph. With the vials lying horizontally, the presence of gelled material is evident when the upper portion of the vial is translucent. The fully blackenend vials (citrate concentrations between 15 and 100 mM) indicate a solution of superparamagnetic materials was maintained. The two vials on the right (citrate concentration of 5 and 10mM) show the formation of a gel. FIG. 5A further shows the characteristic gel obtained without citrate, or with inadequate citrate (5 and 10 mM citrate).

7.16. BIODEGRADABILITY OF SUPERPARAMAGNETIC FLUIDS

Paramagnetic ferric oxyhydroxides are biodegradable and have long been used for the treatment of anemia. There-fore, the biodegradability of the invention's superparamagnetic fluids was compared with the paramagnetic ferric oxy-
5 hydroxides. The ability of both iron preparations to reverse anemia in rats was utilized as a model. The paramagnetic ferric oxyhydroxide was Imferon, and has dextran attached. The supermagnetic fluid also had dextran attached and was produced as described in Example 7.10.

Weanling rats were divided into four groups of five rats each. Rats in group 1 received a chow containing iron and were sacrificed at weeks 5, 6, 7 and 8 to allow establishment of normal iron (hematocrit) levels in rat tissues. Rats in
10 groups 2, 3 and 4 received an iron deficient diet. Rats in group 2 were also sacrificed at weeks 5, 6, 7, and 8 to allow establishment of normal iron levels in rat tissues. Rats in groups 2, 3 and 4 received an iron deficient diet. After receiving the low iron diet for 5 weeks, rats in groups 3 and 4 received intravenous (tail vein) injections of iron to reverse their ane-mia and restore normal levels. Rats in group 3 received Proferdex, while those in group 4 received the dextranized superparamagnetic fluid. Rats receiving iron were injected with a single dose of 30 mg of iron per kilogram, a sufficient
15 dose to reverse their anemia. The results are presented in Table V.

TABLE V

REVERSAL OF ANEMIA WITH SUPERPARAMAGNETIC IRON OXIDE PARTICLES								
	Hematocrit (1% red cells in whole blood)							
	week 5		week 6		week 7		week 8	
	avg	sd	avg	sd	avg	sd	avg	sd
chow	45.1	1.4	45.2	0.5	46.7	0.9	47.0	0.8
low Fe	28.5	2.6	29.5	2.1	32.7	1.2	34.8	2.3
Imferon			43.1	1.9	42.8	1.3	46.3	1.2
Invention			44.3	2.3	42.7	1.1	47.6	1.6

It can be seen that the invention's superparamagnetic iron oxide restores normal hematocrits levels in rats as well
35 as the paramagnetic preparation, Imferon.

7.17. SUMMARY OF SUPERPARAMAGNETIC FLUID PROPERTIES

The properties of the superparamagnetic fluids of the invention compared with solutions made with other types of
40 ferric oxide are summarized TABLE VI:

TABLE VI

SUMMARY OF PROPERTIES OF AQUEOUS SOLUTIONS OF VARIOUS FERRIC OXIDES				
	Mag. Satura-tion (FIG. 4)	MR Relaxiv-ity (Table II)	Biodegrad-ability (Table V)	Citrate reten-tion (Table IV)
FeO:OH	low	none	high	high
gamma Fe_2O_3	high	some		low
Invention	high	high	high	high

Thus, the superparamagnetic fluids of this invention posess a unite combination of magnetic, biological and anion retaining properties.

**7.18. EXTENSION OF THE SERUM LIFETIME OF DEXTRAN COATED SUPERPARAMAGNETIC IRON OXIDE PAR-
TICLES**

To assess the effectiveness of dextran-coated paramagnetic iron oxide as a serum lifetime extender for dextran-
5 coated superparamagnetic iron oxide particles, a comparative study was conducted.

In both trials, a rat of about 300 g was injected with 1 mg Fe/kg body weight of dextran-coated superparamagnetic
metal oxide produced as described in Example 7.1. However, in one trial, the rat was also injected with 2.5 mg Fe/kg
dextran-coated paramagnetic iron oxide (produced following the procedure described in example 7.1 except that no
divalent salt was used) 15 minutes prior to receiving the superparamagnetic material. The T_2 of the subject's blood was
10 measured periodically over the subsequent 3 hours. The results are present in Figure 7.

Briefly, in both trials, the blood T_2 dropped dramatically within 5 minutes after the superparamagnetic material was
added. However, the value rapidly returned to normal in the rat which did not receive the superparamagnetic material,
presumably due to absorption of the agent by the reticuloendothelial system (RES). In contrast, when the paramagnetic
agent was used the T_2 depression is dramatically extended. This is due to a competition between the superparamag-
15 netic and the paramagnetic material for RES receptors, greatly expanding the lifetime of the superparamagnetic agent.

Claims

1. A method for preparing a stable superparamagnetic fluid, the pH of which is between 6-9, comprising:
20
    (i) forming superparamagnetic metal oxide particles by base precipitation of divalent and trivalent metal salts:
    (ii) dispersing and oxidizing said superparamagnetic metal oxide particles by sonication at a temperature
    between ambient temperature and 100°C in order to produce a superparamagnetic fluid;
    (iii) centrifuging said superparamagnetic fluid and retaining the supernatant; and
25   (iv) dialyzing the supernatant in the presence of a polycarboxylate buffer at a pH between 6-9.

2. The method of claim 1 wherein the divalent and trivalent metal salts are iron salts which are present in a ratio rang-
ing from 1:4 to 4:1.

30 3. The method of claim 2 wherein the divalent and trivalent iron salts are $FeCl_2$ and $FeCl_3$.

4. The method of claim 2 or 3 which further comprises replacing a portion of the divalent iron salt with a divalent salt
of cobalt or manganese.

35 5. The method of claim 4 wherein the portion of divalent iron salt replaced is half or less.

6. The method of claim 1-5 wherein said sonication is accomplished by means selected from the group consisting of
a sonic probe and a continuous flow sonicator.

40 7. The method of claim 1-6 wherein said polycarboxylate buffer is selected from the group consisting of a citrate
buffer, a succinate buffer, a maleate buffer and a tartrate buffer.

8. A composition comprising a stable superparamagnetic fluid, said composition being obtainable by the steps com-
prising :
45
    i) forming superparamagnetic metal oxide particles by base precipitation of divalent and trivalent metal salts :
    (ii) dispersing and oxidizing said superparamagnetic metal oxide particles by sonication at a temperature
    between ambient temperature and 100° C in order to produce a superparamagnetic fluid ;
    (iii) centrifuging said superparamagnetic fluid and retaining the supernatant which comprises superparamag-
50   netic particles, each of which:

        (a) comprises one or more individual superparamagnetic metal oxide crystals of $50\text{-}500 \cdot 10^{-10}$ m in diam-
        eter; and
        (b) has an overall mean diameter of $50\text{-}5000 \cdot 10^{-10}$ m in diameter;
55
    and
    (iv) dialyzing the supernatant in the presence of a polycarboxylate buffer at a pH between 6-9.

9. The composition of claim 8 wherein said particles are biodegradable, as evidenced by their ability to be metabolized by a subject within 30 days of their administration.

10. The composition of claim 8 or 9 in which said particles are coated with a polymeric coating.

11. The composition of claim 10 in which said polymeric coating has a molecular weight between 5,000 and 250,000 daltons.

12. The composition of claim 10 or 11 in which said polymeric coating is selected from the group consisting of a carbohydrate, a protein, a polypeptide and an organosilane.

13. The composition of claim 12 in which said carbohydrate is a dextran.

14. The composition of claim 13 in which said dextran is selected from the group consisting of a dextran of 17,900 daltons, a dextran of 35,600 daltons, a dextran of 71,000 daltons and a dextran of 249,000 daltons.

15. The composition of claim 12 in which said protein is albumin.

16. The composition of claim 15 in which said albumin is selected from the group consisting of human serum albumin and bovine serum albumin.

17. The composition of claim 12 in which said polypeptide is selected from the group consisting of polyglutamate and polylysine.

18. The composition of claim 12 in which said organosilane is N-2-aminoethyl-3-aminopropyltrimethoxysilane.

19. The composition of claims 10-12 which further comprises a carbohydrate biomolecule which is covalently coupled to said polymeric coating and which posses functional groups which are capable of facilitating the recognition of the particles by receptors of various organs or tissues upon the particles' administration to a subject.

20. The composition of claims 8-19 in which said polycarboxylate buffer is selected from the group consisting of a citrate buffer, a tartrate buffer, a succinate buffer and a maleate buffer.

21. The composition of claim 20 in which said citrate buffer is derived from the salt selected from the group consisting of sodium citrate, potassium citrate and ammonium citrate.

22. The composition of claims 8-21 in which said metal oxide is an iron oxide.

23. The composition of claim 22 in which:

(i) the concentration of the iron of the iron oxide ranges from 0.05 to 5 molar;
(ii) the polycarboxylate counterion of said polycarboxylate buffer is present in a concentration ranging from 0.001 to 0.1 moles of polycarboxylate counterion per mole of iron; and
(iii) the pH of said fluid is within the range of 6 to 8.3.

24. An improved method for obtaining an in vivo MR image of an organ or tissue of an animal or human subject, in which the improvement comprises administrating to such subject an image enhancing amount of a MR imaging contrast agent, said agent comprising the superparamagnetic fluid of claims 8-23.

25. The method of claim 24 in which said contrast agent is administered by the method selected from the group consisting of intravascular injection, oral administration, intubation and enema.

26. The method of claim 24 in which the organ to be imaged is selected from the group consisting of the gastrointestinal tract, the reticuloendothelial system and the vascular system.

27. The method of claim 26 in which said organ or tissue of the gastrointestinal tract is selected from the group consisting of the esophagus, the small intestine and the large intestine.

28. The method of claim 26 in which said organ or tissue of the reticuloendothelial system is selected from the group consisting of the liver, spleen, lymph nodes, bone marrow, neural tissue and lung.

29. A pharmaceutical composition comprising the composition of claims 8-23.

30. The pharmaceutical composition of claim 29 for use as an in vivo NMR imaging contrast agent.

31. The pharmaceutical composition of claim 29 for use in reducing anemia in an animal or human subject.

32. The pharmaceutical composition of claim 31 which comprises an effective amount of a fluid comprising biodegradable superparamagnetic iron oxide particles and a polycarboxylate buffer, wherein said iron oxide particles :

    a) are comprised of one or more individual biodegradable superparamagnetic iron oxide crystals;
    b) have an overall mean diameter of 50 to 5000 $\cdot$ 10^{-10} m;
    c) are capable of being biodegraded as evidenced by the ability of the iron of said iron oxide particles to be incorporated into the hemoglobin of a subject within 30 days of administration;
    d) are capable of acting as cations in solution; and
    e) are stabilized by the polycarboxylate buffer at a pH between 6-9 based on the attractive forces between the cationic iron oxide particles and the polycarboxylate anions of the polycarboxylate buffer.

33. The pharmaceutical composition of claim 32 in which said particles are coated with a polymeric coating.

Patentansprüche

1. Ein Verfahren zur Herstellung eines stabilen superparamagnetischen Fluids, dessen pH-Wert zwischen pH 6 und pH 9 liegt, die nachgenannten Verfahrensschritte umfassend:

    (i) Bilden von superparamagnetischen Metalloxidpartikeln durch basisches Ausfällen von zweiwertigen und dreiwertigen Metallsalzen:
    (ii) Dispergieren und Oxidieren der besagten superparamagnetischen Metalloxidpartikel durch Beschallen mit Ultraschall bei einer Temperatur zwischen Umgebungstemperatur und 100°C, um ein superparamagnetisches Fluid hervorzubringen;
    (iii) Zentrifugieren des besagten superparamagnetischen Fluids und Zurückbehalten des Überstandes; und
    (iv) Dialysieren des Überstandes in Gegenwart eines Polycarboxylatpuffers mit einem pH-Wert zwischen pH 6 und pH 9.

2. Das Verfahren gemäß Anspruch 1, dadurch gekennzeichnet, daß es sich bei den zweiwertigen und dreiwertigen Metallsalzen um Eisensalze handelt, die in einem Verhältnis zwischen 1:4 und 4:1 vorliegen.

3. Das Verfahren gemäß Anspruch 2, dadurch gekennzeichnet, daß es sich bei den zweiwertigen und dreiwertigen Eisensalzen um $FeCl_2$ und $FeCl_3$ handelt.

4. Das Verfahren gemäß Anspruch 2 oder 3, dadurch gekennzeichnet, daß es desweiteren den Verfahrensschritt des Austauschens eines Teils des zweiwertigen Eisensalzes gegen ein zweiwertiges Kobalt- oder Mangansalz umfaßt.

5. Das Verfahren gemäß Anspruch 4, dadurch gekennzeichnet, daß der ausgetauschte Eisensalz-Anteil die Hälfte oder weniger als die Hälfte ausmacht.

6. Das Verfahren gemäß den Ansprüchen 1 bis 5, dadurch gekennzeichnet, daß die besagte Beschallung mit Hilfe einer Ultraschall-Vorrichtung erfolgt, ausgewählt aus der Gruppe umfassend einen Schallkopf und ein Durchfluß-Ultraschallgerät.

7. Das Verfahren gemäß den Ansprüchen 1 bis 6, dadurch gekennzeichnet, daß der besagte Polycarboxylatpuffer ausgewählt wird aus der Gruppe umfassend einen Citratpuffer, einen Succinatpuffer, einen Maleatpuffer und einen Tartratpuffer.

8. Eine Verbindung, die ein stabiles superparamagnetisches Fluid enthält und die sich anhand der nachgenannten Verfahrensschritte gewinnen läßt:

(i) Bilden von superparamagnetischen Metalloxidpartikeln durch basisches Ausfällen von zweiwertigen und dreiwertigen Metallsalzen;

(ii) Dispergieren und Oxidieren der besagten superparamagnetischen Metalloxidpartikel durch Beschallen mit Ultraschall bei einer Temperatur zwischen Umgebungstemperatur und 100°C, um ein superparamagnetisches Fluid hervorzubringen;

(iii) Zentrifugieren des besagten superparamagnetischen Fluids und Zurückbehalten des Überstandes, der superparamagnetische Partikel enthält, von denen ein jeder:

(a) ein oder mehrere einzelne superparamagnetische Metalloxidkristalle mit einem Durchmesser zwischen 50 und 500 . 10^{-10} m enthält; und

(b) einen mittleren Gesamtdurchmesser zwischen 50 und 5000 . 10^{-10} m aufweist;

und

(iv) Dialysieren des Überstandes in Gegenwart eines Polycarboxylatpuffers mit einem pH-Wert zwischen pH 6 und pH 9.

9. Die Verbindung gemäß Anspruch 8, dadurch gekennzeichnet, daß die besagten Partikel biologisch abbaubar sind, wie ihre Fähigkeit, von einem Subjekt innerhalb von 30 Tagen nach Verabreichung abgebaut zu werden, beweist.

10. Die Verbindung gemäß Anspruch 8 oder 9, dadurch gekennzeichnet, daß die Partikel mit einer Polymerbeschichtung versehen sind.

11. Die Verbindung gemäß Anspruch 10, dadurch gekennzeichnet, daß die besagte Polymerbeschichtung ein Molekulargewicht zwischen 5.000 und 250.000 Dalton aufweist.

12. Die Verbindung gemäß Anspruch 10 oder 11, dadurch gekennzeichnet, daß die besagte Polymerbeschichtung ausgewählt wird aus der Gruppe bestehend aus einem Kohlenhydrat, einem Protein, einem Polypeptid und einem Organosilan.

13. Die Verbindung gemäß Anspruch 12, dadurch gekennzeichnet, daß es sich bei dem besagten Kohlenhydrat um ein Dextran handelt.

14. Die Verbindung gemäß Anspruch 13, dadurch gekennzeichnet, daß das besagte Dextran ausgewählt wird aus der Gruppe bestehend aus einem Dextran mit 17.900 Dalton, einem Dextran mit 35.600 Dalton, einem Dextran mit 71.000 Dalton und einem Dextran mit 249.000 Dalton.

15. Die Verbindung gemäß Anspruch 12, dadurch gekennzeichnet, daß es sich bei dem besagten Protein um Albumin handelt.

16. Die Verbindung gemäß Anspruch 15, dadurch gekennzeichnet, daß das besagte Albumin ausgewählt wird aus der Gruppe bestehend aus Serumalbumin vom Menschen und Serumalbumin vom Rind.

17. Die Verbindung gemäß Anspruch 12, dadurch gekennzeichnet, daß das besagte Polypeptid ausgewählt wird aus der Gruppe bestehend aus Polyglutamat und Polylysin.

18. Die Verbindung gemäß Anspruch 12, dadurch gekennzeichnet, daß es sich bei dem besagten Organosilan um N-2-Aminoethyl-3-aminopropyltrimethoxysilan handelt.

19. Die Verbindung gemäß den Ansprüchen 10 bis 12, dadurch gekennzeichnet, daß sie desweiteren ein Biomolekül enthält, das kovalent an die besagte Polymerbeschichtung gebunden ist und das funktionelle Gruppen besitzt, die in der Lage sind, die Erkennung der Partikel durch Rezeptoren verschiedener Organe oder Gewebe nach Verabreichung der Partikel an ein Subjekt zu erleichtern.

20. Die Verbindung gemäß den Ansprüchen 8 bis 19, dadurch gekennzeichnet, daß der besagte Polycarboxylatpuffer ausgewählt wird aus der Gruppe bestehend aus einem Citratpuffer, einem Tartratpuffer, einem Succinatpuffer und einem Maleatpuffer.

21. Die Verbindung gemäß Anspruch 20, dadurch gekennzeichnet, daß besagter Citratpuffer abgeleitet wird aus dem aus der Gruppe bestehend aus Natriumcitrat, Kaliumcitrat und Ammoniumcitrat ausgewählten Salz.

22. Die Verbindung gemäß den Ansprüchen 8 bis 21, dadurch gekennzeichnet, daß es sich bei dem besagten Metalloxid um ein Eisenoxid handelt.

23. Die Verbindung gemäß Anspruch 22, dadurch gekennzeichnet, daß:

   (i) die Eisenkonzentration des Eisenoxids zwischen 0,05 und 5 mol liegt;
   (ii) das Polycarboxylat-Gegenion des besagten Polycarboxylatpuffers im einer Konzentration zwischen 0,001 und 0,1 mol Polycarboxylat-Gegenion pro mol Eisen vorliegt; und
   (iii) der pH-Wert des besagten Fluids zwischen pH 6 und pH 8,3 liegt.

24. Ein verbessertes Verfahren zur Gewinnung eines nach dem Kernspinresonanz-(MR-)Verfahren aufgenommenen In-vivo-Bildes eines tierischen oder menschlichen Organs oder Gewebes, dadurch gekennzeichnet, daß die Verbesserung die Verabreichung einer bildverbessernden Dosis eines Kernspintomographie-(MRI-)Kontrastmittels an das Tier oder den Menschen beinhaltet, wobei das Kontrastmittel das superparamagnetische Fluid gemäß den Ansprüchen 8 bis 23 enthält.

25. Das Verfahren gemäß Anspruch 24, dadurch gekennzeichnet, daß das besagte Kontrastmittel nach der aus der Gruppe bestehend aus intravaskulärer Injektion, oraler Verabreichung, Sondierung und Einlauf gewählten Methode verabreicht wird.

26. Das Verfahren gemäß Anspruch 24, dadurch gekennzeichnet, daß das bildlich darzustellende Organ ausgewählt wird aus der Gruppe bestehend aus dem Magendarmtrakt, dem reticulo-endothelialen System und dem vaskulären System.

27. Das Verfahren gemäß Anspruch 26, dadurch gekennzeichnet, daß das besagte Organ oder Gewebe des Magendarmtrakts ausgewählt wird aus der Gruppe bestehend aus der Speiseröhre, dem Dünndarm und dem Dickdarm.

28. Das Verfahren gemäß Anspruch 26, dadurch gekennzeichnet, daß das besagte Organ oder Gewebe des reticulo-endothelialen Systems ausgewählt wird aus der Gruppe bestehend aus der Leber, der Milz, den Lymphknoten, dem Knochenmark, dem Nervengewebe und der Lunge.

29. Eine pharmazeutische Verbindung, die die Verbindung gemäß den Ansprüchen 8 bis 23 enthält.

30. Die pharmazeutische Verbindung gemäß Anspruch 29 zur Verwendung als ein zur Bilderzeugung nach dem Kernspinresonanz-Verfahren in vivo eingesetztes Kontrastmittel.

31. Die pharmazeutische Verbindung gemäß Anspruch 29 zur Verwendung zum Zwecke der Anämie-Abschwächung bei einem Tier oder einem Menschen.

32. Die pharmazeutische Verbindung gemäß Anspruch 31 umfassend eine wirksame Menge eines Fluids, das biologisch abbaubare superparamagnetische Eisenoxidpartikel sowie einen Polycarboxylatpuffer enthält, dadurch gekennzeichnet, daß besagte Eisenoxidpartikel:

   a) aus ein oder mehreren einzelnen biologisch abbaubaren superparamagnetischen Eisenoxidkristallen bestehen;
   b) einen mittleren Gesamtdurchmesser zwischen 50 und 5.000 . 10^{-10} m aufweisen;
   c) sich biologisch abbauen lassen, wie die Inkorporierbarkeit des Eisens der besagten Eisenoxidpartikel in das Hämoglobin eines Subjekts innerhalb von 30 Tagen nach Verabreichung beweist;
   d) in der Lage sind, in Lösung als Kationen zu wirken; und
   e) durch den Polycarboxylatpuffer mit einem pH-Wert zwischen pH 6 und pH 9 auf Grund der zwischen den kationischen Eisenoxidpartikeln und den Polycarboxylatanionen des Polycarboxylatpuffers wirkenden Anziehungskräfte stabilisiert werden.

33. Die pharmazeutische Verbindung gemäß Anspruch 32, dadurch gekennzeichnet, daß besagte Partikel mit einer Polymerbeschichtung versehen sind.

Revendications

1. Un procédé pour préparer un fluide super-paramagnétique stable, dont le pH se situe entre 6 et 9, comportant:

(i) la formation de particules en oxyde métallique super-paramagnétique par précipitation au moyen d'une base de sels divalents et trivalents;

(ii) la dispersion et l'oxydation desdites particules en oxyde métallique super-paramagnétique par sonication à une température entre la température ambiante et 100°C afin d'obtenir un fluide super-paramagnétique;

(iii) la centrifugation dudit fluide super-paramagnétique et la conservation de la phase surnageante; et

(iv) la dialyse de la phase surnageante en présence d'un tampon au polycarboxylate à un pH entre 6 et 9.

2. Le procédé de la revendication 1, dans lequel les sels de métaux, divalents et trivalents, sont des sels de fer qui sont présents selon un rapport se situant dans la gamme de 1:4 à 4:1.

3. Le procédé de la revendication 2 dans lequel les sels de fer, divalent et trivalent sont $FeCl_2$ et $FeCl_3$.

4. Le procédé de la revendication 2 ou 3, comporte en outre, le remplacement d'une partie du sel de fer divalent par un sel divalent de cobalt ou de manganèse.

5. Le procédé de la revendication 4, dans lequel la partie du sel de fer divalent est remplacé à moitié ou moins.

6. Le procédé des revendications 1 à 5, dans lequel ladite sonication est réalisée par des moyens choisis dans le groupe constitué d'une sonde sonique et d'un sonicateur à écoulement continu.

7. Le procédé des revendications 1 à 6, dans lequel ledit tampon au polycarboxyl est choisi dans le groupe constitué d'un tampon au citrate, d'un tampon au succinate, d'un tampon au maléate et d'un tampon au tartrate.

8. Une composition comportant un fluide stable super-paramagnétique, cette composition pouvant être obtenue par les étapes comportant:

(i) la formation de particules en oxyde métallique super-paramagnétique par précipitation au moyen d'une base de sels divalents et trivalents;

(ii) la dispersion et l'oxydation desdites particules en oxyde métallique super-paramagnétique par sonication à une température entre la température ambiante et 100°C afin d'obtenir un fluide super-paramagnétique;

(iii) la centrifugation dudit fluide super-paramagnétique et la conservation de la phase surnageante qui comporte des particules super-paramagnétiques dont chacune :

(a) comporte un ou plus d'un cristal individuel en oxyde métallique super-paramagnétique de 50 à 500.10^{-10}m de diamètre, et

(b) présentant un diamètre moyen global de $50-5000.10^{-10}$m; et

(iv) la dialyse de la phase surnageante en présence d'un tampon au polycarboxylate à un pH entre 6 et 9.

9. La composition de la revendication 8, dans laquelle les particules sont biodégradables comme mis en évidence par leur capacité à être métabolisées par un sujet dans les 30 jours de leur administration.

10. La composition de la revendication 8 ou 9, dans laquelle lesdites particules sont enrobées au moyen d'un revêtement polymère.

11. La composition de la revendication 10, dans laquelle ledit revêtement polymère représente un poids moléculaire entre 5000 et 250.000 daltons.

12. La composition de la revendication 10 ou 11, dans laquelle ledit revêtement polymère est choisi dans le groupe constitué d'un hydrate de carbone, d'une protéine, d'un polypeptides et d'un organosilane.

13. La composition de la revendication 10, dans laquelle ledit hydrate de carbone est un dextrane.

14. La composition de la revendication 13, dans laquelle ledit dextrane est choisi dans le groupe constitué par un dextrane de 17.900 daltons, un dextrane de 35.600 daltons, un dextrane de 71.000 daltons et un dextrane de 249.000 daltons.

15. La composition de la revendication 12, dans laquelle ladite protéine est l'albumine.

16. La composition de la revendication 15, dans laquelle ladite albumine est choisie dans le groupe constitué de la sérum albumine humaine et de la sérum albumine de bovin.

17. La composition de la revendication 12, dans laquelle ledit peptide est choisi dans le groupe constitué du polygluta-mate et de la polylysine.

18. La composition de la revendication 12, dans laquelle ledit organosilane est le N-2-aminoéthyl-3-aminopropyltrimé-thoxysilane.

19. La composition des revendications 10 à 12, qui comporte en outre une molécule de type hydrate de carbone qui est couplée de façon covalente audit revêtement polymère et qui possède des groupes fonctionnels qui sont aptes à faciliter la reconnaissance des particules par des récepteurs de différents organes ou tissus après l'administra-tion des particules à un sujet.

20. La composition des revendications 8 à 19, dans laquelle ledit tampon au polycarboxylate est choisi dans le groupe constitué d un tampon au citrate, d'un tampon au tartrate, d'un tampon au succinate, et d'un tampon au maléate.

21. La composition de la revendication 20, dans laquelle le tampon au citrate est dérivé du sel choisi dans le groupe constitué du citrate de sodium, du citrate de potassium et du citrate d'ammonium.

22. La composition des revendications 8 à 21, dans laquelle ledit oxyde métallique est un oxyde de fer.

23. La composition de la revendication 22, dans laquelle:

(i) la concentration du fer et de l'oxyde de fer se situe de 0,05 à 5 M;
(ii) le contre-ion de type polycarboxylate dudit tampon au polycarboxylate est présent dans uns concentration se situant dans la gamme de 0,0001 à 0,1 mole de contre-ion au polycarboxylate mole de fer; et
(iii) le pH dudit fluide se situe dans la gamme de 6 à 8,3.

24. Un procédé amélioré pour obtenir une image RM in vivo d'un organe ou d'un tissu d'un sujet animal ou humain, dans lequel l'amélioration consiste à administrer audit sujet une quantité améliorant l'image d'un agent contraste de visualisation RM, ledit agent comportant le fluide super-paramagnétique des revendications 8 à 23.

25. Le procéde de la revendication 24, dans lequel ledit agent de contraste est administré par une technique choisie dans le groupe constitué d'une injection intra-vasculaire, d'une administration orale, d'une intubation et d'un clys-tère.

26. Le procédé de la revendication 24, dans lequel l'organe devant être visualisé est choisi dans le groupe constitué du tractus gastro-intestinal, du système réticulo-endothélial et du système vasculaire.

27. Le procédé de la revendication 26, dans lequel ledit organe ou tissu du tractus gastro-intestinal est choisi dans le groupe constitué de l'oesophage, du petit intestin et du gros intestin.

28. Le procédé de la revendication 26, dans lequel ledit organe ou tissu du système réticulo-endothélial est choisi dans le groupe constitué dit foie, de la rats, des ganglions lymphatiques, de la moelle osseuse, du tissu nerveux et du poumon.

29. Une composition pharmaceutique comportant la composition des revendications 8 à 23.

30. La composition pharmaceutique de la revendication 29 destinée à être utilisée comme agent de contraste pour visualisation RMN in vivo.

31. La composition pharmaceutique de la revendication 29 destinée à être utilisée pour la réduction de l'anémie chez un sujet animal ou humain.

32. La composition pharmaceutique de la revendication 31, qui comporte une quantité efficace d'un fluide comportant des particules biodégradables en oxyde de fer super-paramagnétique et un tampon en polycarboxylate, dans laquelle lesdites particules en oxyde de fer:

a) sont constituées d'un ou plus d'un cristal individuel biodégradable en fer super-paramagnétique;

b) présentent un diamètre moyen global de 50 à 5000.10^{-10}m;

c) sont aptes à être biodégradés comme cela est mis en évidence par l'aptitude du fer desdites particules en oxyde de fer à être incorporés dans l'hémoglobine d'un sujet pendant les 30 jours d'administration.

d) sont aptes à agir en tant que cation dans la solution; et

e) sont stabilisées par le tampon au polycarboxylate à un pH entre 6 et 9, en se basant sur les forces d'attraction entre les particules cationiques d'oxyde de fer et les anions polycarboxylate du tampon au polycarboxylate.

33. La composition pharmaceutique de la revendication 32, dans laquelle lesdites particules sont enrobées d'un revêtement polymère.

FIG. 1

Superparamagnetic

Pf − 2228

1/T2, Sec⁻¹

Fe³⁺ mM
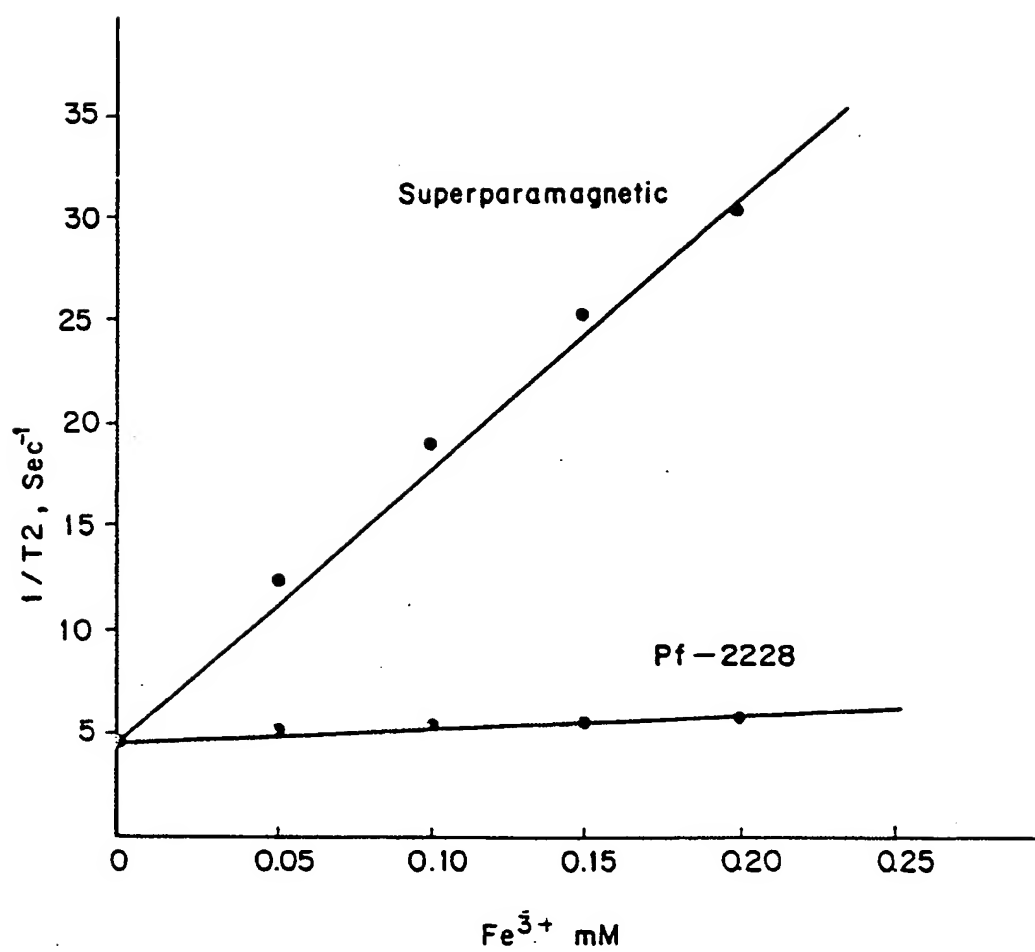
FIG. 2A   FIG. 2B

FIG. 2C   FIG. 2D

FIG. 2E

FIG. 3

FIG. 4

FIG. 5A

5mM   10mM

FIG. 5B

100mM   50mM   25mM   15mM   10mM   5 mM
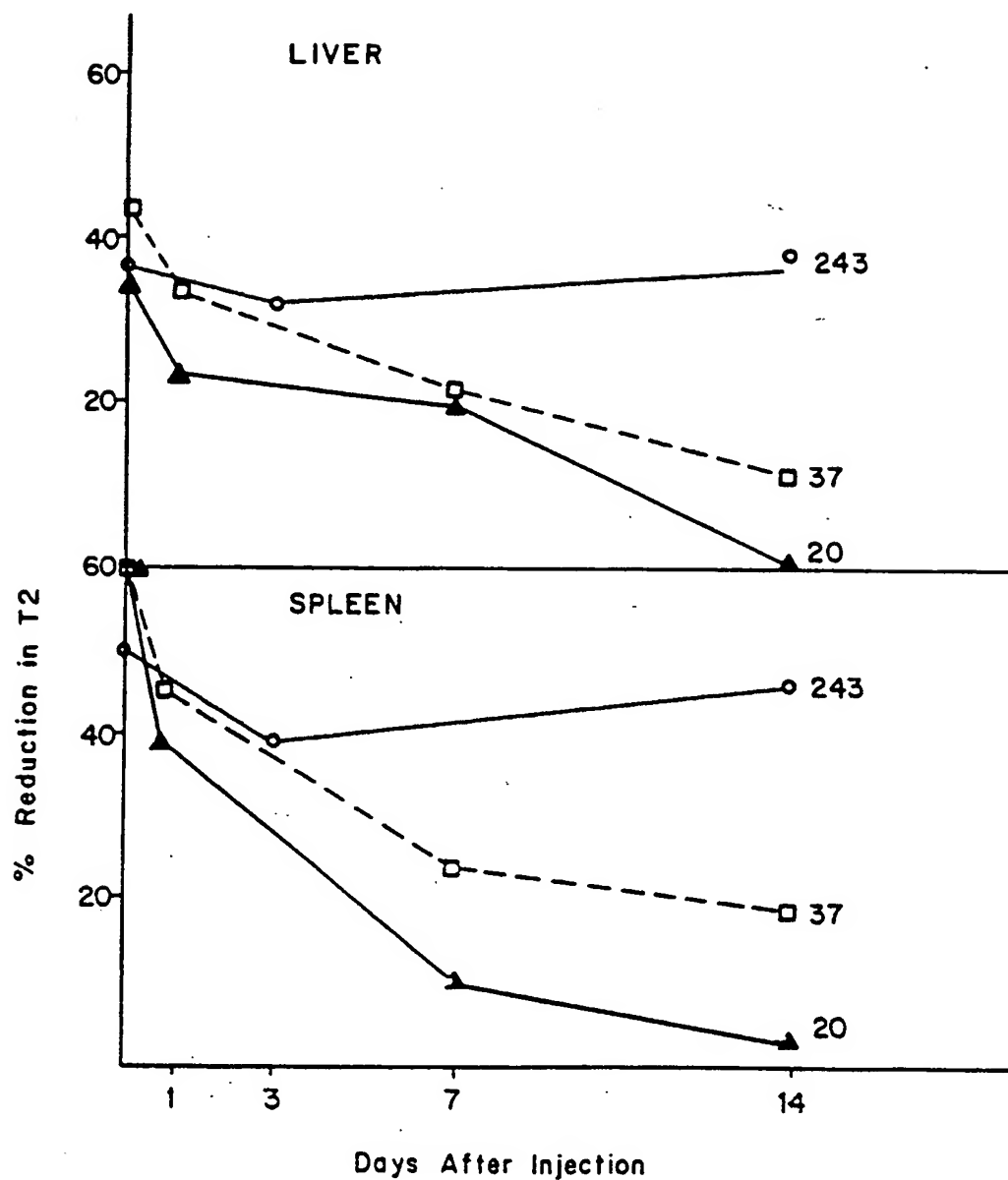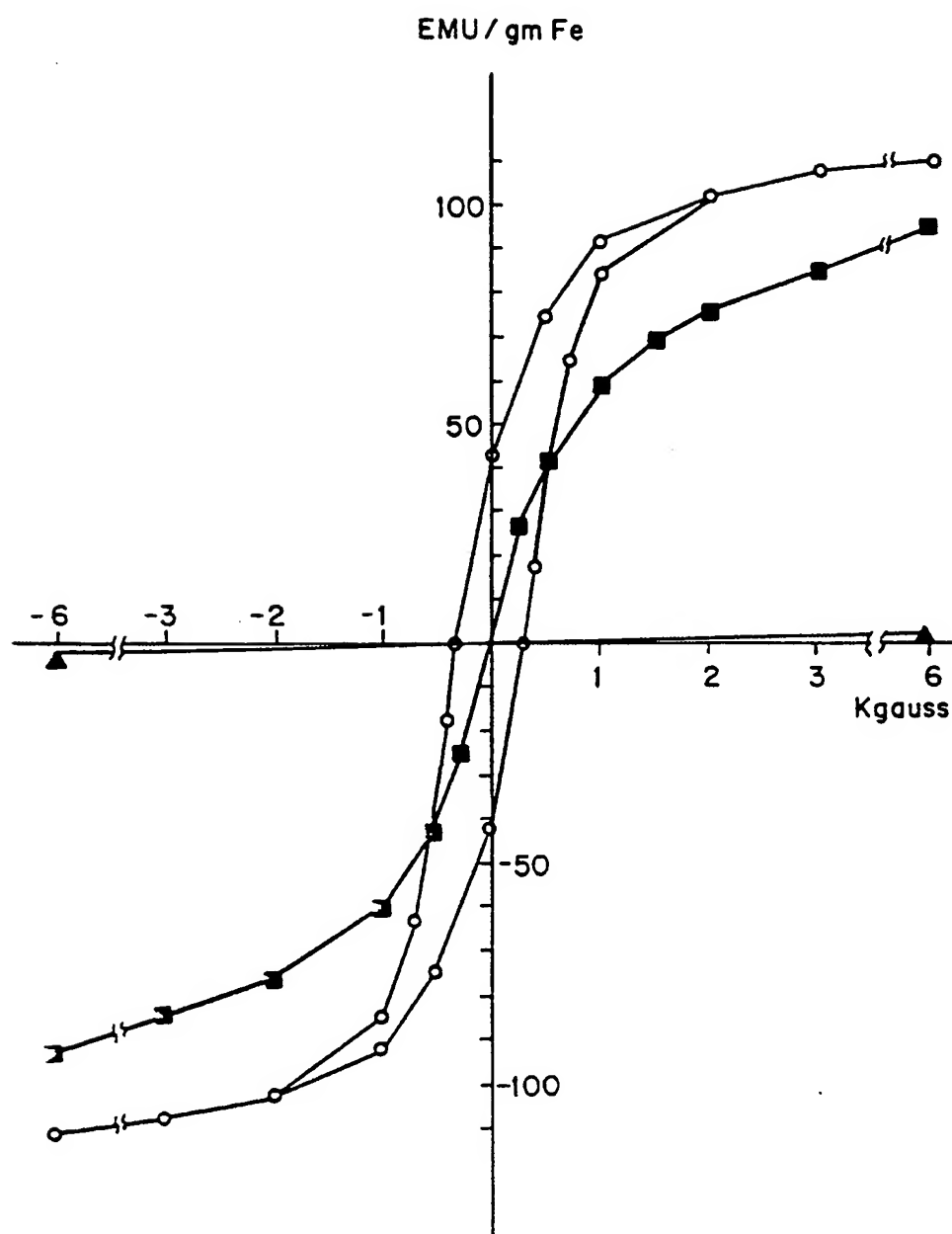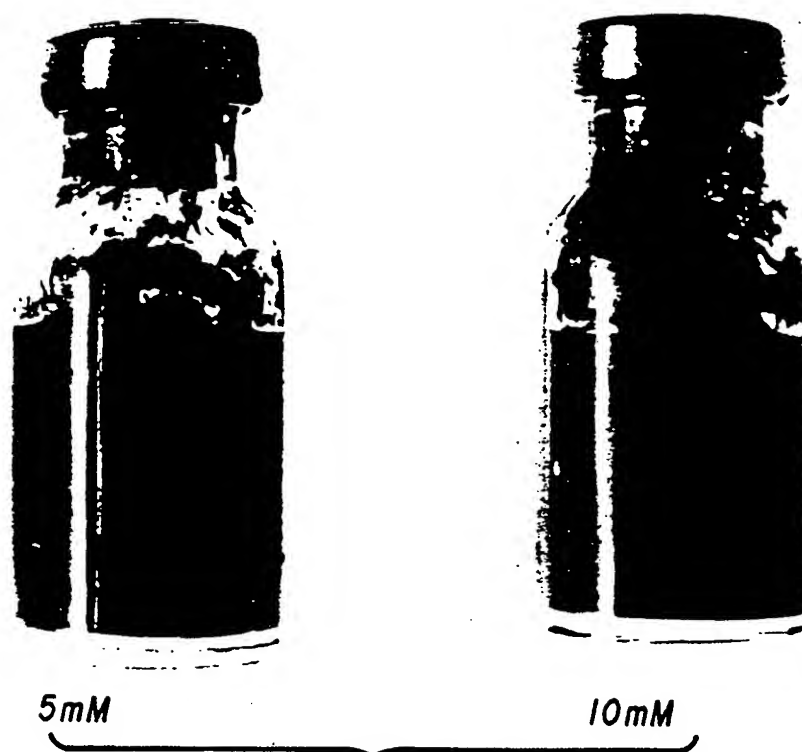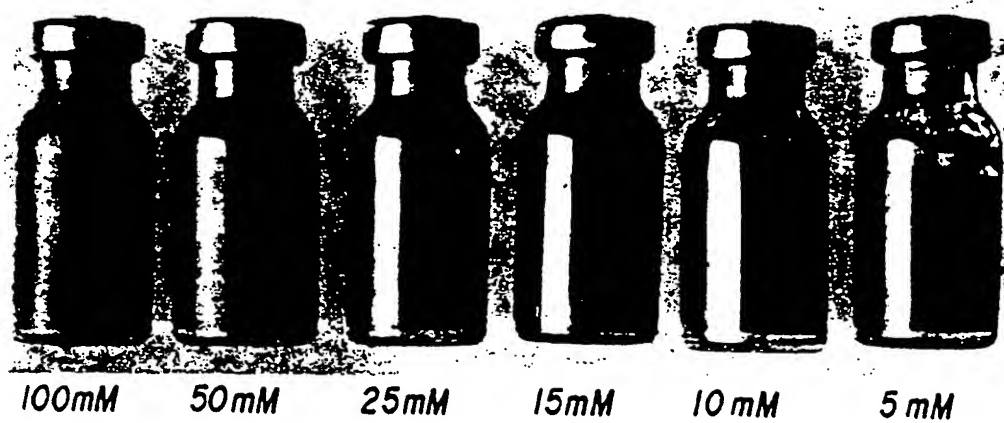
FIG. 6

Schematic Diagram of Apparatus For Synthesizing Stable
Superparamagnetic Fluids

Pump
Sonicator
Heating Coil
(100° C)
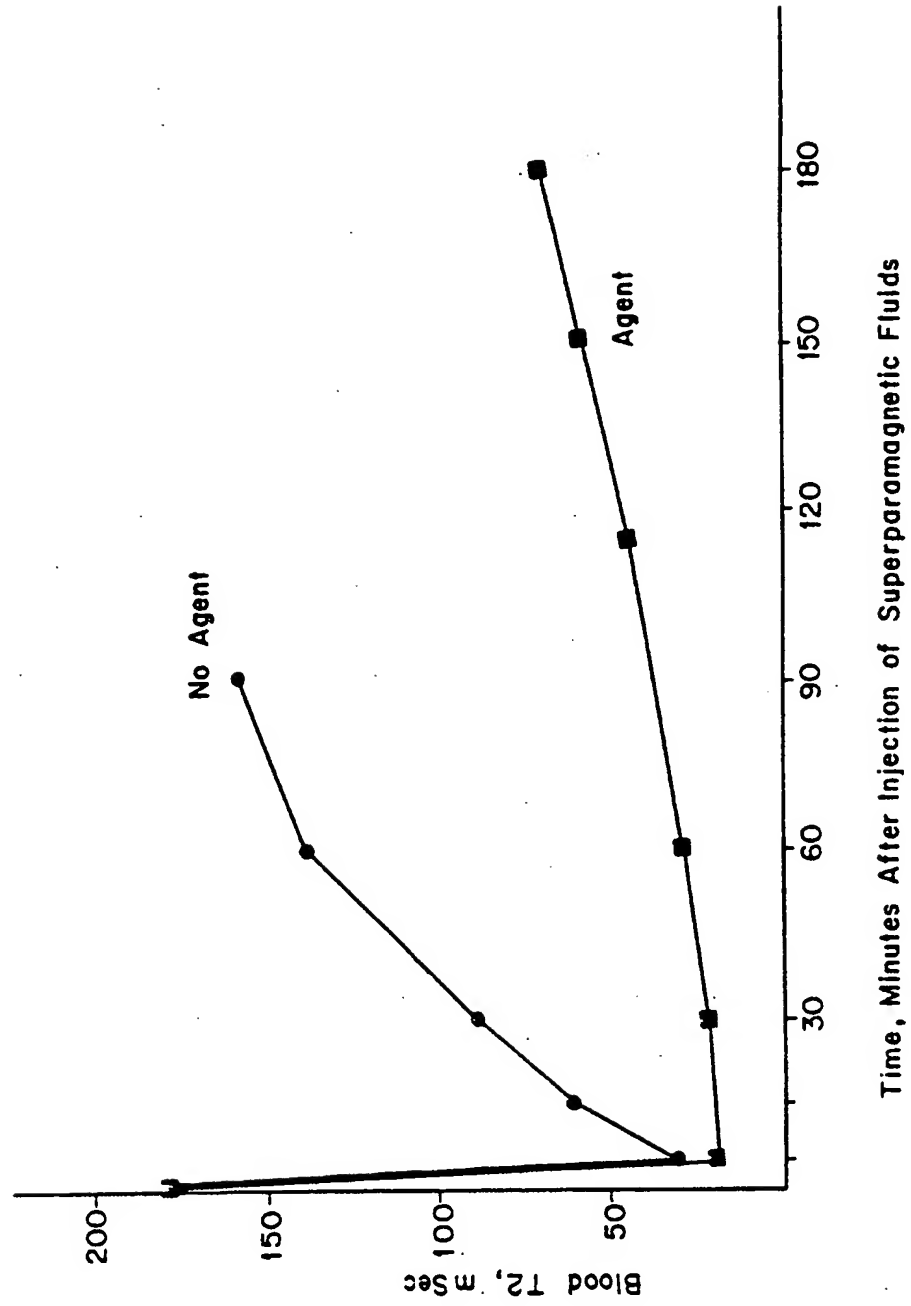Cooling Coil
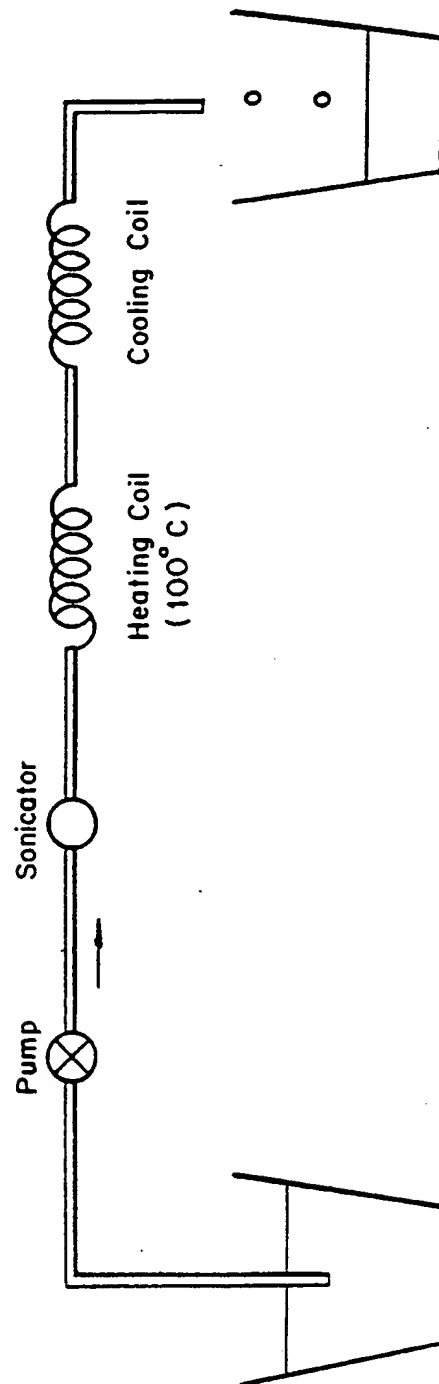
FIG. 7 Blood T2 Depression With Blocking Agent

No Agent

Agent

Blood T2, mSec

200
150
100
50

30
60
90
120
150
180

Time, Minutes After Injection of Superparamagnetic Fluids